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SYNTHESIS OF 2,5-DIDEOXY-5-*C*-METHYLSTREPTAMINE

BY

© TAKAHIRO HAGA

A THESIS

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The undersigned certify that they had read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SYNTHESIS OF 2,5-DIDEOXY-5-*C*-METHYLSTREPTAMINE submitted by TAKAHIRO HAGA in partial fulfilment of the requirements for the degree of Master of Science.

To my wife Yoshiko, for her patience and understanding during the preparation of this manuscript.

ABSTRACT

It has been previously shown in this laboratory that the equatorial substituent in the 2'-position of the cyclohexyl aglycon of an α-glucoside plays an extremely important role in determining the population on each conformer. These results suggested that, by removal of hydroxyl group on 5-position of streptamine part of kanamycin and changing it into methyl group, a relation between biological activity and chemical structure may be obtained.

As the first step of the total synthesis of such 5-position analogs of kanamycin, the synthesis of 2,5-dideoxy-5-*C*-methylstreptamine was examined. The direct diepoxidation of 3-methyl-1,4-cyclohexadiene proved not useful. However, monohydroxybromination of 3-methyl-1,4-cyclohexadiene in aqueous dioxane gave as the main product, *arabino*-3-methyl-4-bromo-5-hydroxycyclohexene and epoxidation of this compound gave mainly the epoxide *trans* to the methyl group. Therefore, it was possible to achieve the synthesis of *trans*,*trans*-1,4-diepoxy-3-*C*-methylcyclohexane from which, following Suami's procedure for the preparation of 2,5-dideoxystreptamine, 2,5-dideoxy-5-*C*-methylstreptamine could be prepared. However, the compound could not be separated from a minor (~20%) amount of the 5-epimer which has the methyl group in the axial orientation.

ACKNOWLEDGEMENT

The author expresses his appreciation to Professor R. U. Lemieux for his guidance, invaluable advice and understanding during the course of this work. He is grateful for the excellent research facilities provided by the University of Alberta.

The author also extends his appreciation to his company, Ishihara Company Limited, Japan, who gave him the opportunity to study chemistry in Canada and to his colleagues in Professor Lemieux's group for their guidance.

Lastly, and most importantly, the author wishes to thank the Canadian people for their friendliness that has made his stay in Canada a very enjoyable experience.

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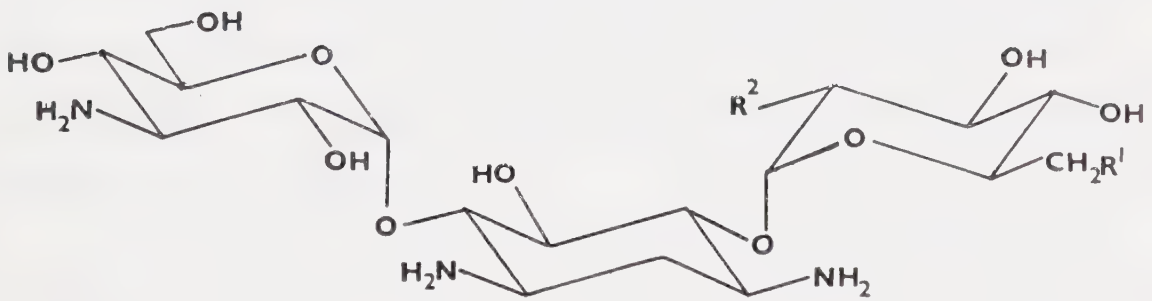
I. INTRODUCTION

Kanamycin A, discovered by Umezawa *et al* (1) in 1957, is an antibiotic active against a variety of the Gram-negative bacteria, especially against the mycobacteria, and now widely used as an anti-tuberculous agent. The structure was demonstrated independently by Japanese and American-Canadian groups (2-5) and the points of attachment to the deoxystreptamine residue were established by Rinehart, Tatsuoka and their associates (6). The compound has the structure 1a, in which 6-amino-6-deoxy-D-glucose and 3-amino-3-deoxy-D-glucose are linked in the α-form at C-4 and C-6 positions, respectively, of the 2-deoxy-streptamine moiety. Closely related structures kanamycin B (1b) and kanamycin C (1c) are formed along with kanamycin A in the fermentation broth of *Streptomyces kanamyceticus*.



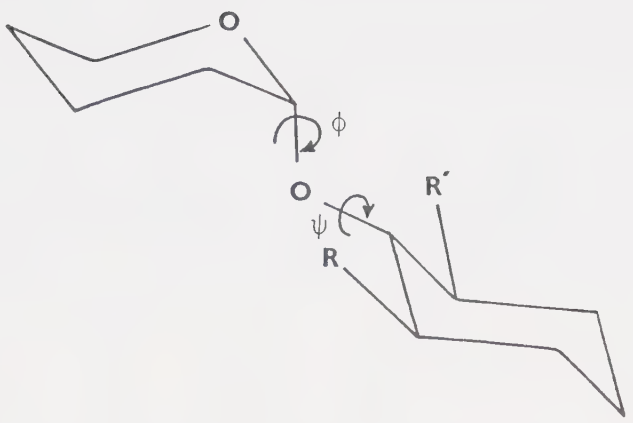
(1a) $R^1 = NH_2$, $R^2 = OH$

(1b) $R^1 = R^2 = NH_2$

(1c) $R^1 = OH$, $R^2 = NH_2$

This project was initiated as the first stage of an investigation of the role of the 5-hydroxyl group in determining the biological activity of a kanamycin. Such an investigation seemed pertinent since it is well established that the antibiotic activity of a kanamycin is related to interactions involving the amino groups. This is clearly apparent from many observations which show that alterations of the amino groups nearly always lead to near complete loss of activity. The one notable exception is the semi-synthetic kanamycin A derivative known commercially as Amikacin (7). On the other hand, many deoxy-derivatives of kanamycin A have high activity (8-10). The exact mode of action of kanamycin A is not known but may be related to the special distributions of the amino groups. Thus, it can be imagined that the amino groups must be distributed in space in such a manner that the protonated structure has a strong and specific affinity for a polyanionic structure such as a nucleic acid at the ribosome level of the microorganism and thereby lead to a change in metabolism lethal to the organism.

The conformational preferences for glycosidic linkages have been recently reviewed by Lemieux and Koto (11). On the basis of hard-sphere type calculations, it was demonstrated that changes in the bulk of the R and R' groups (structure 2) in the aglycon portion of an α-glycopyranoside can be expected to lead to substantial changes in the ϕ and ψ torsion angles for the glycosidic

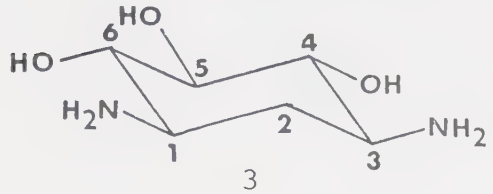


2

linkage. The ϕ angle is expected to be near ±60° for reasons of the *exo*-anomeric effect. However, other values for the ϕ torsion angle may occur as the most favorable compromise between non-bonded interactions and the *exo*-anomeric effect.

Thus, it could be expected that a change in either the hydration and/or the steric bulk of the 5-substituent of the deoxystreptamine residue of a kanamycin could influence appreciably the overall conformation of the molecule and thereby have an important effect on the antimicrobial activity of the compound. Therefore, it seemed of interest to plan syntheses of such kanamycin analogs. Such syntheses could be envisaged since Nakajima (12) and Umezawa and coworkers (13-15) have reported syntheses of the kanamycins and the methods developed in these labora-

tories should in principle apply to syntheses starting with a deoxystreptamine (3) modified at the 5-position.



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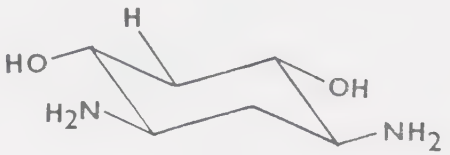

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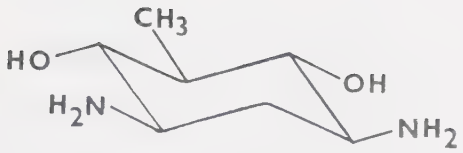

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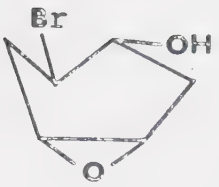
At the beginning of this investigation, it was planned to achieve syntheses of both 2,5-dideoxystreptamine (4) and 2,5-dideoxy-5-*C*-methylstreptamine (5). Soon thereafter, Suami and coworkers (16) published an extremely elegant synthesis of 4 starting from commercially available 1,4-cyclohexadiene. Therefore, it was decided to model a synthesis of 5 on this approach.

Craig, Harvey and Berchtold, in 1967, had published the synthesis of *cis*-1,4-diepoxy-cyclohexane (7) by peracid oxidation of the monobromohydrin derivative of the diene to form 6 in high yield followed by treatment of 6 with base (17).



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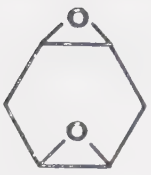

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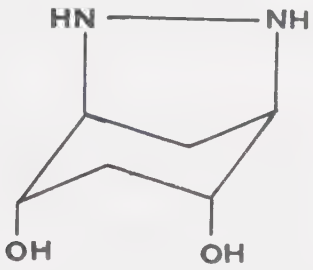
Suami and coworkers conceived of reacting 7 with hydrazine to form the cyclic hydrazino compound 8 which on hydrogenolysis should yield the desired 2,5-dideoxy-streptamine (4).



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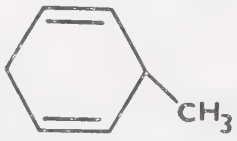
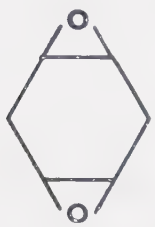


9

In view of the success of the method, it was decided to attempt the synthesis of 2,5-dideoxy-5-*C*-methylstreptamine (5) from the known 3-methyl-1,4-cyclohexadiene (9). Two approaches could be envisaged: (a) the direct diepoxidation of 9 and (b) the epoxidation of a monobromohydrin of 9.

Although the diepoxidation of 1,4-cyclohexadiene is known (17) to lead almost exclusively to the *trans*-diepoxide

10, the stereochemical result of a similar diepoxidation of 9 could not be anticipated with certainty. Presumably, the first epoxidation would lead mainly to compound 11 which has the epoxide ring *trans* to the methyl group.



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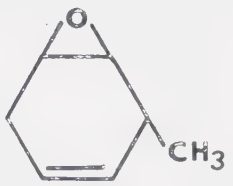

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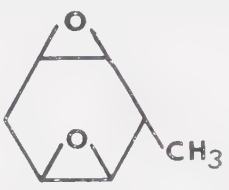
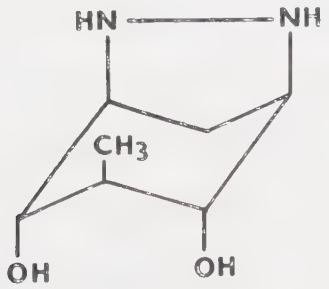


12

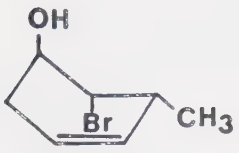
Conceivably, the methyl group would have the dominating influence on the direction for the formation of the second epoxide ring and the desired diepoxide 12 would be achieved.

The structure of the cyclic hydrazino compound formed on treatment of 12 with hydrazine would depend on whether the initial attack by the hydrazine was at the 1- or 2-position. Assuming that the attack occurs at the less hindered 1-position, then the overall course of reaction would lead to the formation of the desired intermediate 13.



13

The second approach would require in the first instance that monohydroxybromination would yield a bromohydrin with the bromine atom in *cis*-relationship to the methyl group (either 14 or 15). Formation of either 16 or 17 in high yield would render this approach without value.



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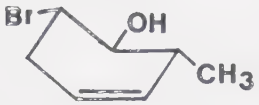

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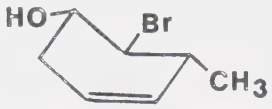

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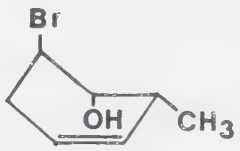

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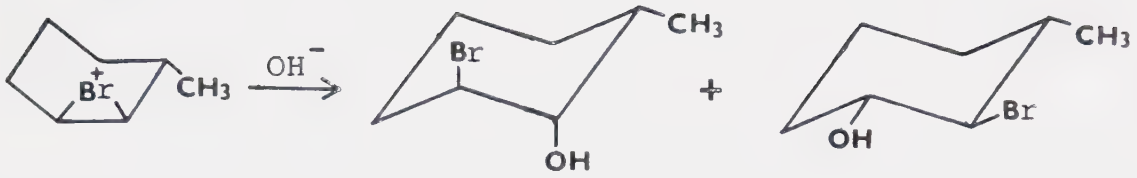
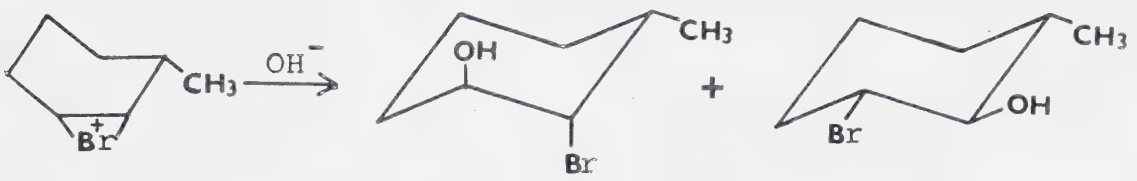
An indication that the monohydroxybromination of the 3-methyl-1,4-cyclohexadiene (9) would tend to yield structures 14 and 15 was found in the recent work reported by Barili and coworkers (18) on the hydroxybromination of 3-methylcyclohexene using *N*-bromosuccinimide. It was found that whereas bromination by way of the *trans*-bromonium ion 18 to form products 19 and 20 was favored in weakly polar media, the reverse occurred to place the bromine in *cis* relation to the methyl group in a polar aqueous medium. That is, such conditions favored formation of the *cis*-bromonium ion 21 and, as a consequence, the formation of 22 and 23 which have the bromine atoms in *cis*-relationship to the methyl group. The major product was 22 as would be expected on the basis of the Fürst-Plattner rule (19) for the opening of 1,2-epoxide groups by way of nucleophilic attack on carbon.



18 19 20



21 22 23

This thesis then comprises of a study of reactions of 3-methyl-1,4-cyclohexadiene (9) in an effort to establish a synthetic route for 2,5-dideoxy-5-*C*-methylstreptamine (5). It will be seen that the work was indeed successful but the study was terminated prior to the achievement of a sample of 5 free of contamination by the 5-epimer wherein the methyl group is in axial orientation.

II. EXPERIMENTAL

A. Materials

1. Solvents

Except for dichloromethane, pyridine, methanol, dimethylformamide and tetrahydrofuran , reagent grade solvents were used without further purification. The solvents which were purified were treated following standard methods (20).

All solvent removals were performed using a rotary evaporator at temperatures less than 45°C.

2. Reagents

The palladium-on-charcoal (5%) and the platinum-on-carbon (5%) were purchased from Terochem Laboratories Ltd., Edmonton, Alberta and Engelhard Industries, Newark, New Jersey, respectively.

The *p*-toluenesulfonyl chloride was purified by recrystallization from chloroform-Skellysolve B according to Fiesers' method (21).

The monoperphthalic acid was prepared just before using by treating recrystallized phthalic anhydride with aqueous sodium perborate solution followed by extraction with ether (22).

B. Analytical Methods

All melting points were determined in capillary tubes using a Gallenkamp melting point apparatus or Ernst Leitz

melting point apparatus and are uncorrected.

The elemental analyses were performed in this department.

Spectroscopic measurements

Unless otherwise noted, the solvent used to measure nuclear magnetic resonance (nmr) spectra was $CDCl_3$. Proton magnetic resonance (pmr) spectra were recorded on a Varian A-60 and HA-100 spectrometer. Chemical shifts are reported in delta (δ) values from tetramethylsilane (TMS). Proton noise-decoupled carbon-13 natural abundance nuclear magnetic resonance (cmr) spectra were recorded using a Bruker HFX-10 spectrometer (Model 221-1605), at ambient temperature, with a Nicolet Fourier transform system. The chemical shifts are from TMS.

Infrared (ir) spectra were recorded on a Perkin-Elmer grating spectrophotometer (Model 421), at ambient temperature, using matched sodium chloride cells.

All spectra were determined by the spectral services laboratories of this department.

Chromatographic procedures

Thin layer chromatography (tlc) was performed on Silica Gel G supplied by E. Merck A.G., Darmstadt, W. Germany, using microscopic slides. Different solvent systems were chosen according to the compounds as noted in the text. The compounds were visualized by spraying with 5% sulfuric acid in ethanol and heating on a hot plate.

Column chromatography was carried out using Silica Gel G in the ratio of 30 g/g of compound at a flow rate of 1 ml per min. The developing solvents for individual compounds are specified in the text.

Descending paper chromatography was performed on Whatman No. 1 paper using *n*-butanol-pyridine-water-acetic acid (6:4:3:1) as the developing solvent and the components were visualized by spraying with ninhydrin solution followed by heating at 100°C.

C. Synthetic Investigations

3-Carboxy-1,4-cyclohexadiene (24)

Ammonia gas was introduced into a 5-liter reaction flask which was set in the solid carbon dioxide-acetone vessel and 2.5 liter of liquid ammonia was collected. Benzoic acid (85 g, 0.67 mol) in 250 ml of methanol was then slowly added and this was followed by the addition of sodium metal (50 g) in small portions under a nitrogen atmosphere. The blue coloration, which first appeared, disappeared quickly. After the addition of ammonium chloride (400 g) in 600 ml of cold water, the ammonia was removed under vacuum. Ether (1 liter) was then added. The reaction mixture was acidified with 20% hydrochloric acid at -20°C to obtain a clear solution which was twice extracted with ether. The combined extracts were washed with water and then dried over magnesium sulfate. On evaporation of the ether, 79.3 g (95.5%) of oil was obtained:

Plieninger and Ege (23) reported a yield of 90% and mp -20°C. The pmr spectrum was now measured and found to be in accord with the structure assignment.

Pmr data for 24: δ 2.56-2.79 (multiplet, two H-6); δ 3.61-3.84 (triplet, H-3); δ 5.87 (near singlet, olefinic protons, H-1, H-2, H-4, and H-5).

3-Hydroxymethyl-1,4-cyclohexadiene (25)

To a slurry of 84 g of lithium aluminum hydride in 1,500 ml of ether, a solution of 140 g of 24 in 1,000 ml of ether was added dropwise with stirring at such a rate as to cause gentle refluxing of the ether. The mixture was heated under reflux for an additional hr, cooled to 0°C and hydrolyzed by the cautious addition of water (250 ml) followed by 20% by weight aqueous sulfuric acid (1,700 ml). The ether extract was washed with water, dried over sodium sulfate and distilled. The yield was 124.6 g (83%), boiling point, 84-86°C/18 mmHg. lit. (24), 95-97°C/20 mmHg. The following pmr parameters are in accord with the structure assignment.

Pmr data: δ 1.75 (singlet, H-3); δ 2.75 (broad singlet, two H-6); δ 3.62 (doublet, spacing 7 Hz, *exo*-methylene protons); δ 5.52-6.16 (multiplet, four olefinic protons, H-1, H-2, H-4, and H-5); δ 7.37 (singlet, hydroxy proton).

3-*p*-Toluenesulfonyloxymethyl-1,4-cyclohexadiene (26)

To a solution of 25 (110 g) in 1,500 ml of anhydrous pyridine, *p*-toluenesulfonyl chloride (190.5 g, 1.0 mol) was added with swirling. The stoppered solution was allowed to stand at 0-5°C for 48 hr and then evaporated to near dryness. The mixture was twice extracted with ether. The combined extracts were sequentially washed with water, ice-cold *N* hydrochloric acid, dilute sodium bicarbonate solution, and water. After drying over sodium sulfate followed by the evaporation of ether, a light yellow oil was freed of volatile components by use of a high vacuum. The yield was 237 g (90%). The pmr and cmr spectra reproduced in Fig. 1 are in accord with the structure assigned by Nelson *et al* (24).

Pmr data: δ 2.45 (singlet, 3H); δ 2.56-2.77 (broad multiplet, 2H); δ 2.80-3.20 (broad multiplet, 1H); δ 3.92 (doublet, 9 Hz, 2H); δ 5.37-6.02 (multiplet, 4H); δ 7.24-7.93 (multiplet, 4H).

Cmr data: δ 21.58, 26.33, 35.50, 73.42, 123.86 (2C), 127.10 (2C), 127.96 (2C), 129.80 (2C), 133.52 and 144.63.

Anal. Calcd. for $C_{14}H_{15}O_3S$: C, 63.80; H, 6.12. Found: C, 63.35; H, 6.03.

3-Methyl-1,4-cyclohexadiene (9)

To a rapidly stirred suspension of lithium aluminum hydride (15 g) in 800 ml of anhydrous ether, a solution of 26 (103 g, 0.4 mol) in 300 ml of ether was added. The

mixture was refluxed overnight. After cooling in an ice-water bath, the mixture was treated successively with 13 ml of water, 13 ml of 30% sodium hydroxide solution, and 40 ml of water. The insoluble material was gathered by filtration and was washed with ether. The combined ether solution was distilled to give 18 g of 9, boiling point, 75-78°C/20 mmHg. Distillation under atmospheric pressure caused the decomposition of the product and therefore the boiling point of the preparation could not be compared with that (102.5-103°C) reported in the literature (25). The yield was 50.9% (lit. 61.2%). The pmr and cmr spectra reproduced in Fig. 2 are in accord with the structure assigned by Paquette *et al* (25).

Pmr data: δ 1.08 (doublet, 7.2 Hz, 3H); δ 2.54-2.92 (multiplet, 3H); δ 5.63 (singlet 4H).

Cmr data: δ 22.44, 26.11, 30.16, 123.27 (2C), and 130.82 (2C).

Direct diepoxidation of 3-methyl-1,4-cyclohexadiene (9)

3-Methyl-1,4-cyclohexadiene (470 mg, 5 mmol) was added to an ether solution containing 20 mmol of monoperphthalic acid and the mixture was kept overnight in the dark at room temperature. The solution was filtered and washed with aqueous sodium carbonate and saturated aqueous sodium chloride. Drying over sodium sulfate followed by the evaporation of ether afforded 560 mg (90%) of an oil which showed three spots on tlc (developing solvent, acetone: benzene = 1:4) with Rf values of 0.65, 0.50 and 0.33. The

product was applied to a silica gel column using the same solvent system for chromatographic separation into the three components which were characterized by pmr spectroscopy as shown in Fig. 3.

The pmr spectrum of the first major component (210 mg, 38%) reproduced in Fig. 3A required it to be monoepoxide since the material still showed a signal of olefinic hydrogen.

The second fraction was also major (230 mg, 36%) and the Rf value of this fraction on tlc showed the same Rf (0.50) as *trans*-diepoxide (10) of 1,4-cyclohexadiene. The pmr spectrum is reproduced in Fig. 3B.

The third fraction was minor (40 mg, 6%) and the Rf value was similar to that of *cis*-diepoxide (7) of 1,4-cyclohexadiene. No signal for olefinic hydrogen was observed and instead multiplet appeared in the epoxide ring proton region 2.8-3.1 ppm on the pmr spectrum reproduced in Fig. 3C.

Pmr data for the first fraction: δ 1.04-1.40 (doublets, 7 Hz, 3H); δ 2.10-3.10 (multiplet, 3H); δ 3.20-4.60 (multiplet, 2H); δ 5.40 (near singlet, 2H).

Pmr data for the second fraction: δ 1.12 (doublet, 7 Hz, 3H); δ 2.27 (triplet, 1H); δ 2.40 (quartet, 1H); δ 2.77 (broad singlet, 1H); 2.90-3.13 (multiplet, 4H).

Pmr data for the third fraction: δ 1.18 (doublet, 7 Hz, 3H); δ 1.37 (doublet, 7 Hz); δ 2.28 (triplets of doublet,

17.0 and 3.0 Hz, 1H); δ 2.67 (broad singlet, 1H); δ 2.76-3.13 (multiplet, 5H).

5-Bromo(or hydroxy)-4-hydroxy(or bromo)-3-*C*-methylcyclohexene (14-17)

To 9 (8 g, 84 mmol) in 34 ml of water and 3.4 ml of dioxane, N-bromosuccinimide (14.2 g, 84 mmol) was added in portions with stirring. After 3 hr, the reaction was extracted with chloroform. The extract was successively washed with saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride. The solution was dried over sodium sulfate. Concentration left 14.3 g (77%) of a colorless oil which was shown to be the mixture of 4 isomers by both thin layer chromatography (developing solvent acetone:benzene = 1:4) and pmr. The pmr spectrum reproduced in Fig. 5 is in accord with the structure expected.

Pmr data: δ 1.01-1.34 (four doublets, 7 Hz, 3H); δ 2.00-2.94 (multiplet, 3H); δ 2.34 (singlet, -OH); δ 3.62-4.38 (multiplet, 2H); δ 5.37-5.58 (multiplet, 2H).

Cmr data: δ 18.72 (main), 18.99, 20.28, 32.42 (main), 33.02, 34.09 (main), 36.68, 38.73, 40.68, 58.15, 62.52 (main), 67.06, 67.38 (main), 68.52, 71.26, 75.63, 77.04, 77.63, 78.49, 122.14, 122.84 (main), 123.48, 130.07 (main), 130.66, and 132.00.

Hydrogenation-hydrogenolysis reaction of crude bromohydrin derivatives 14-17 of 3-methyl-1,4-cyclohexadiene

To a solution of crude bromohydrin derivatives (1.0 g, 5 mmol) and triethylamine (1.5 g) in methanol (10 ml), a suspension of 5% palladium-on-charcoal (1.0 g) in methanol (5 ml) prepared in advance was carefully added (26). Then the mixture was hydrogenated in a Parr Reaction Apparatus for 3 hr at room temperature (pressure 50 psi). The reaction product was filtered through Celite and washed with methanol. The combined filtrates were freed of methanol at atmospheric pressure and the residue was extracted with chloroform. The extract was sequentially washed with water, dilute hydrochloric acid and water. Drying over sodium sulfate followed by the evaporation of chloroform at atmospheric pressure afforded 490 mg (89%) of oil.

Pmr data: δ 0.80-1.10 (doublets, 3H); δ 1.20-2.40 (multiplet, 10H); δ 3.50-4.42 (multiplet, 1H).

Cmr data: δ 18.01, 18.13, 18.61, 19.42, 20.01 (main), 22.01 (main), 22.39, 24.28, 25.25, 25.79, 26.60 (main), 30.16, 31.56, 32.91, 33.18 (main), 33.29, 33.39, 33.77, 34.20 (main), 35.50, 41.65 (main), 44.72, 66.89 (main), 70.83, 75.69, 77.09, and 78.49.

1,2-Epoxy-5(or 4)-bromo-3-*C*-methyl-4(or 5)-cyclohexanols (27-34)

The crude bromohydrin mixture (14-17) (14 g, 73.3 mmol) was added to an ether solution containing 100 mmol of monoperphthalic acid and the mixture was kept overnight in the dark at room temperature. The solution was filtered and washed with aqueous sodium carbonate and saturated aqueous sodium chloride. Drying over sodium sulfate followed by the evaporation of ether afforded 12.6 g (83%) of oil which showed two components on tlc (developing solvent, acetone:benzene = 1:4). This mixture was used directly in the preparation of 12.

Pmr data: δ 1.20-1.43 (several doublets, 7 Hz, 3H); δ 2.03-2.80 (multiplet, 3H); δ 3.06-3.40 (narrow multiplet, 2H); δ 3.64-4.38 (multiplet, 2H).

Cmr data: δ 16.99 (main), 17.69, 17.96, 19.34, 20.15, 28.51 (main), 30.08, 31.80 (main), 33.37, 34.50, 36.79, 37.57, 52.09 (main), 56.43 (main), 58.07 (main), 60.04, 60.69, 65.30, 67.43 (main), 68.59, and 70.54.

1,2;4,5-Diepoxy-3-*C*-methylcyclohexane (12 and 35)

A mixture of 27-34 (12.6 g, 60 mmol) in 126 ml of *N* aqueous sodium hydroxide was stirred for 1 hr and extracted with chloroform. The extract was dried over sodium sulfate. Concentration gave 7.6 g (100%) of an oil which was shown to be a mixture of at least three compounds by both tlc (developing solvent, acetone:benzene =

1:4) and nmr. The product was chromatographed on a silica gel column, using acetone:benzene (5:95) as solvent, to yield three fractions; A (1.2 g, 16%), B (1.1 g, 14%) and C (5.0 g, 63%). The nmr parameters for the major component fraction C (crude 12) are given below.

Pmr data for 12: δ 1.18 (doublet, 7 Hz, 3H); δ 2.28 (triplets of doublet, 3 Hz and 17.0 Hz, 1H); δ 2.67-3.13 (multiplet, 6H); δ 1.37 (doublet, 7 Hz, 3H of isomer 35).

Cmr data for 12: δ 15.00, 23.60, 27.40, 48.90 (2C), and 54.27 (2C).

Anal. Calcd. for $C_7H_{10}O_2$: C, 66.64; H, 7.99. Found: C, 64.78; H, 7.38.

3-Methyl-6,7-diazabicyclo[3,2,1]octane-2,4-diols (13 and 36).

Fraction C (the above-termed crude 12, 2.0 g) was mixed with anhydrous hydrazine (5 ml) and 2-methoxyethanol (120 ml). The solution was refluxed for 4.5 hr then evaporated to a crystalline residue which after washing with ethanol afforded 1.3 g (50%) of product. Attempted recrystallization from aqueous ethanol resulted in decomposition. Because of its instability no attempt was made to characterize this product by nmr and it was used directly in the following preparation.

(1,3,5/4,6) and (1,3/4,5,6)-2,5-Dideoxy-5-*C*-methylstreptamine (5 and 37) as dihydrochloride

A solution of the crude product 13 (1.25 g, 7.5 mmol) in a 1:1 mixture of ethanol and water containing 12 M hydrochloric acid (1.2 ml) was hydrogenated in a Parr Pressure Reaction Apparatus in the presence of a platinum catalyst (70 mg) under hydrogen stream (3.4 kg/cm^2) for 4.5 hr at room temperature. The catalyst was removed by filtration through Celite and the filtrate was evaporated to give a crystalline product, which was washed with ethanol, 1.1 g (61%) of 5 (mp > 290°C). The pmr spectrum reproduced in Fig. 12A showed, on examination of the methyl-proton region, that the product contains two isomers (ratio 82:18).

Pmr data in deuterium oxide: δ 1.32 (doublet, 7.6 Hz, 3H); δ 1.62-2.10 (multiplet, 2H); δ 2.63 (triplet of doublets, 14.0 and 3.5 Hz, respectively, 1H); δ 3.28-3.82 (multiplet, 4H); δ 1.12 (doublet, 7.6 Hz, 3H of isomer 37); 4.02 (doublet of doublets, 12.0 Hz and 5.2 Hz, respectively, 2H of isomer 37).

Cmr data in deuterium oxide: δ 13.0, 28.8, 43.1, 52.8 (2C), 73.0 (2C), 29.2 (C-5 of 37), 40.0 (C-2 of 37), 48.9 (C-1 and C-3 of 37), 70.5 (C-4 and C-6 of 37).

Anal. Calcd. for $C_7H_{18}Cl_2N_2O_2$: C, 36.05; H, 7.72; N, 12.01. Found: C, 36.05; H, 7.74; N, 12.30.

Mixture of (1,3,5/4,6) and (1,3/4,5,6)-1,3-bis-phthalimido-5-*C*-methyl-4,6-cyclohexanediol diacetate (38 and 39)

To the solution of 5 (233 mg, 1 mmol) in methanol (5 ml) and pyridine (5 ml) were successively added triethylamine (202 mg, 2 mmol), phthalic anhydride (148 mg, 1 mmol) in triethylamine (202 mg, 2 mmol), and then phthalic anhydride (163 mg, 1.1 mmol). After the reaction was kept at 40°C for 30 min, excess methanol was added to decompose remaining phthalic anhydride. After evaporation of methanol, excess acetic anhydride and pyridine were added and the mixture was kept stirring overnight. The reaction was extracted with chloroform and the extract was washed with water. After drying over sodium sulfate, chloroform was evaporated to obtain 550 mg (nearly 100%) of an oily solid which was purified by column chromatography on silica gel using chloroform as developing solvent (Rf value on tlc, 0.51, developing solvent, methanol:chloroform = 1:9). The product recrystallized from methanol showed a mp, 292-294°C. The pmr spectrum (reproduced in Fig. 13) indicated an 85:15 mixture.

Pmr data: δ 1.04 (doublet, 6 Hz, 3H); δ 1.87 (singlet, 6H); δ 1.72-2.20 (multiplet, 2H); δ 3.80 (quartet, 12, 12 and 12 Hz, 1H); δ 4.27-4.58 (octet, 4, 10.5, and 12.0 Hz, 2H); δ 5.57 (triplet, 11 Hz, 2H); δ 7.65-7.92

(multiplet, 8H); δ 1.18 (doublet, 6 Hz, 3H of isomer 39); δ 5.79-5.96 (small quartet, 11 and 5 Hz, 2H of isomer 39).

Anal. Calcd. for $C_{27}H_{24}N_2O_8$: C, 64.28; H, 4.79; N, 5.55. Found: C, 64.19; H, 4.88; N, 5.51.

D. Characterization of the Mixture of the 2,5-Dideoxy-5-*C*-Methylstreptamine Dihydrochlorides

1. The Pmr and Cmr Spectra of the Mixture

The pmr and cmr spectra for the mixture are reproduced in Figs. 12A and 12B. The relative intensities of the signals for the two *C*-methyl groups in the pmr spectrum were 82:18.

2. Attempted Purifications

(a) Recrystallization was tried from water-ethanol-ether mixed solvent as follows (27). The mixture of 5 and 37 (200 mg) was dissolved in 1 ml of water in 200 ml beaker to which 50 ml of ethanol was added to make a homogeneous solution. Then, the beaker was put in a large desiccator which already contained ether in the bottom part and allowed to stand for 24 hr until a substantial amount of crystals had appeared. The volume increase of the solution was 34 ml. Filtration gave a first crop of 50 mg. The mother liquor was similarly allowed to stand in the desiccator. When the volume increase of the solution reached 35 ml, the crystals were gathered to afford a second crop (85 mg). Evaporation of filtrate gave a third crop (30 mg). The percentage content of axial-methyl isomer (37) in each

crop was estimated by the comparison of the intensities for the methyl proton signals. These were found to be 16% (first crop), 21% (second crop) and 26% (third crop).

(b) Fractional recrystallization starting from 1.20 g of the mixture by above-mentioned recrystallization method was tried according to the Weissberger's scheme (28).

In each recrystallization step the mother liquor was evaporated to dryness to check the weight of the residue. The weight of the crop was obtained by subtracting the weight of the residue from that of each starting mixture. The results are summarized in Fig. 15.

(c) Descending paper chromatography was performed using 1-butanol:pyridine:water:acetic acid = 6:4:3:1 mixture as developing solvent. The Rf value obtained was 0.22 for both isomers 5 and 37. Repetition of development-drying three times on the same Whatman No. 1 paper showed only one spot after treatment with ninhydrin spray reagent.

(d) The separation with ion exchange resin was attempted by using a column of Dowex 1-X8 (OH anion form) which was prepared in advance by treating Dowex 1-X8 (Cl anion form) with *N* NaOH aqueous solution. The mixture (100 mg) was applied to the column and distilled water was used as developing solvent. The flow rate was 1 ml per minute and a hundred 10 ml fractions were collected. Each fraction was checked with ninhydrin spray reagent but no material was recovered because of the adsorption on the resin.

3. Qualitative Analysis by Amino Acid Analyzer

The crude mixture and crop A and crop B were analyzed using a Beckman Model 120C amino acid analyzer by Dr. B. Singh of the Department of Biochemistry using a standard procedure (29) for basic amino acids at pH 5.2 and detection by reaction with ninhydrin.

III. DISCUSSION

As considered in the Introduction, the goal of this investigation was to make available derivatives of 2-deoxystreptamine wherein the modification is at the 5-position; that is, compounds which have the 5-hydroxyl substituted by a different group R. Thus, incorporation of these compounds by chemical synthesis would provide analogs of kanamycin 1 for an assessment of the effect of change at this position on the antibiotic activity. This in turn may be related to the influence of the 5-position on the conformational preferences about the glycosidic bonds at the adjacent positions.

3 R = OH

4 R = H

5 R = CH_3

Soon after this investigation was started, as mentioned in the Introduction, an elegant synthesis of 2,5-dideoxystreptamine was presented by Suami and coworkers (16). This synthesis was based on 1,4-cyclohexadiene as starting material. The possibility of achieving a synthesis of 5 ($R = CH_3$) in a similar manner from 3-methyl-1,4-cyclo-

hexadiene (9) *via* a *cis*-diepoxide derivative was therefore considered. The method assures the concerted 1,3-*cis*-relationship for the two amine groups and, assuming *trans*-opening of the epoxide groups, a 4,6-*cis*-relationship for the two hydroxyl groups which in turn would be *trans* to the two amine groups. The achievement of the appropriate *cis*-diepoxide and the study of its reaction with hydrazine was the main goal of this research.

Prior to undertaking the possible synthesis of 2,5-dideoxy-5-*C*-methylstreptamine (5) (R = CH_3), it was believed desirable to repeat the preparation of 2,5-dideoxystreptamine (4) (R = H) following the procedures reported by Suami and coworkers (16). There was no difficulty in reproducing the results reported and, starting with 50 g of commercial 1,4-cyclohexadiene, the five-step synthesis yielded 11 g (8% overall yield) of the 2,5-dideoxystreptamine as the dihydrochloride salt. As will be seen later on, the nuclear magnetic resonance spectra of the various compounds were useful to the present study.

The first stage in the synthesis of 2,5-dideoxy-5-*C*-methylstreptamine (5) was to achieve 3-methyl-1,4-cyclohexadiene (9). This compound cannot be obtained by a Birch reduction of toluene since the weakly electronegative methyl group directs the reaction to exclusive formation of 2-methyl-1,4-cyclohexadiene (30). However, the reduction of benzoic acid with sodium in liquid ammonia is known (23) to provide 3-carboxy-1,4-cyclohexadiene (24) and this

compound was used as starting material. The preparation, following the literature directions, afforded no problem and the product was found, as expected, to give three groups of signals in the pmr spectrum of relative intensities 4:1:2 centered at 5.87, 3.78 and 2.67 ppm, respectively. The low field signal for the olefinic hydrogen was a *pseudo* singlet indicating little difference of chemical shift between the 1,5 and 2,4 hydrogens and weak coupling with other hydrogens. The signal for the methine hydrogen at the 3-position and methylene group were complex bands.

The plan was to reduce the carboxyl group to methyl group as previously reported by Nelson *et al* (24) and Paquette *et al* (25). The first step involves reduction of the carboxyl group of 24 to the hydroxymethyl group by lithium aluminum hydride. The experience was similar to that reported independently by the two American groups and the product (25) displayed a boiling point range near that reported (24). Since the characterization reported in the literature involved only a mention of the boiling point range, the pmr spectrum in $CDCl_3$ was determined and found to be in accord with the structure assignment.

The second step was to tosylate 25 to give 3-*p*-toluenesulfonyloxymethyl-1,4-cyclohexadiene (26) following the procedure reported by Nelson *et al* (24). Both the pmr and cmr spectra for 26 as seen in Fig. 1 were in accord with the structure and, therefore, the material was reduced with lithium aluminum hydride to obtain desired compound 9.

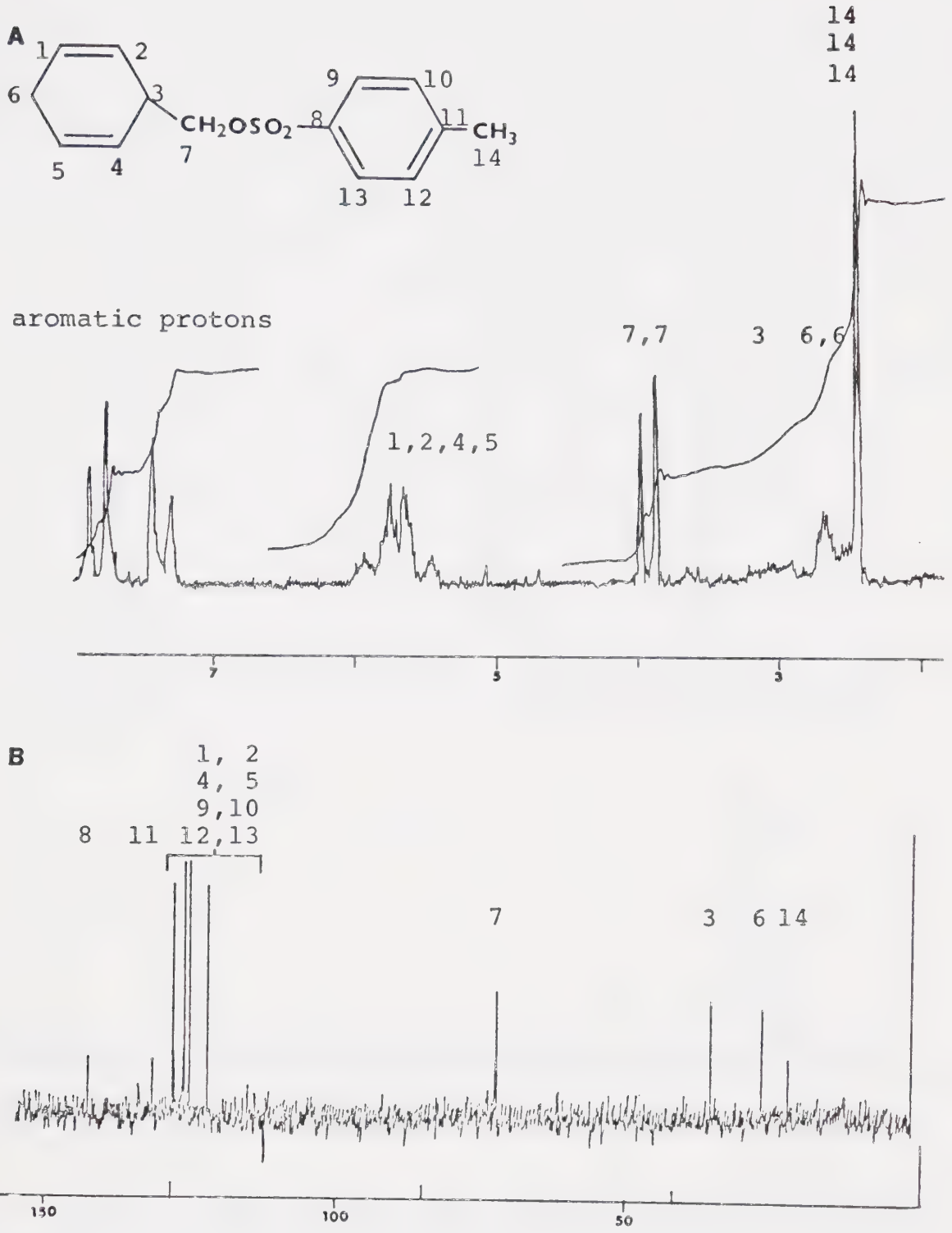


Fig. 1. Nmr spectra for 3-*p*-toluenesulfonyloxymethyl-1,4-cyclohexadiene (26). The assignments are based on the above numbering for the various carbon atoms.

A. 60 MHz pmr spectrum in $CDCl_3$.

B. 22.6 MHz cmr spectrum in $CDCl_3$.

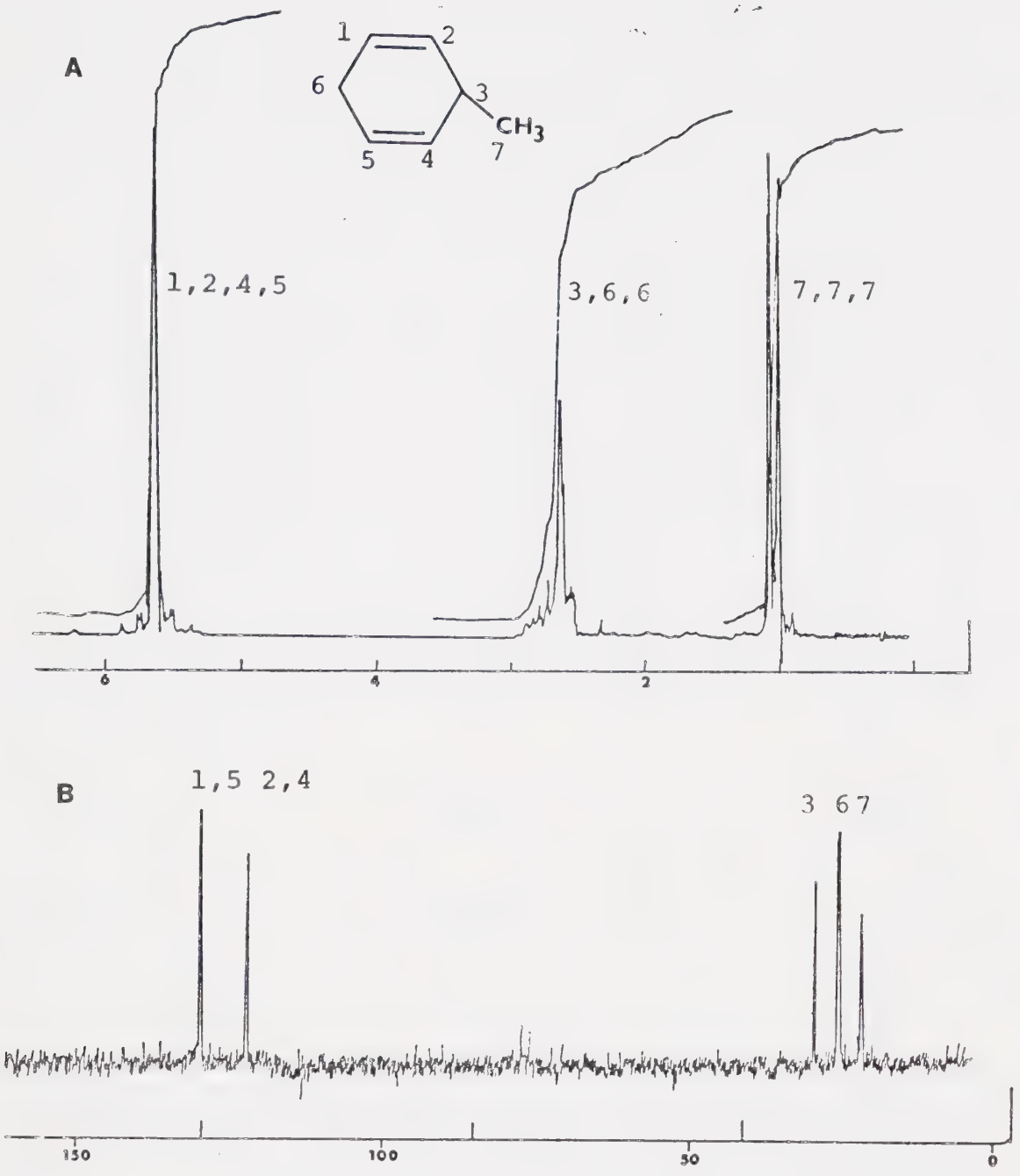


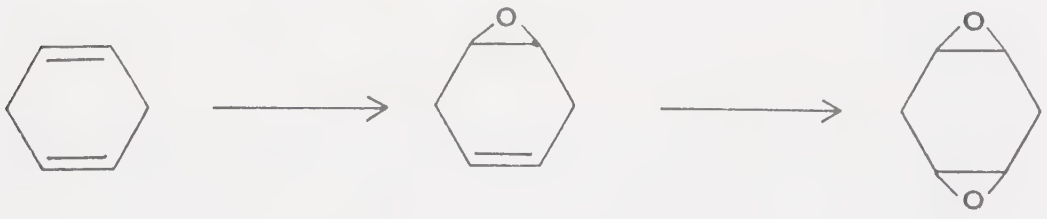
Fig. 2. Nmr spectra for 3-methyl-1,4-cyclohexadiene (9). The assignments are based on the above numbering for the various carbon atoms.

A. 100 MHz pmr spectrum in $CDCl_3$.

B. 22.6 MHz cmr spectrum in $CDCl_3$.

The pmr and cmr spectra (Fig. 2) of the crude product were in accord with expectation. The cmr spectrum especially required the product to be essentially pure and the compound was used as such in the following preparations.

The second stage in the synthesis of 2,5-dideoxy-5-*C*-methylstreptamine (5) was to achieve *trans*, *trans*-1,2; 4,5-diepoxy-3-*C*-methyl-cyclohexane (12). First of all, the direct diepoxidation was considered. It is established that 1,4-cyclohexadiene is a near flat structure (31) and 3-methyl-1,4-cyclohexadiene (9) can be expected to have a similar conformation. It is known that, in the case of 1,4-cyclohexadiene, direct diepoxidation with excess monoperphthalic acid gives rise to only the *trans*-epoxide 10 as shown below (17).



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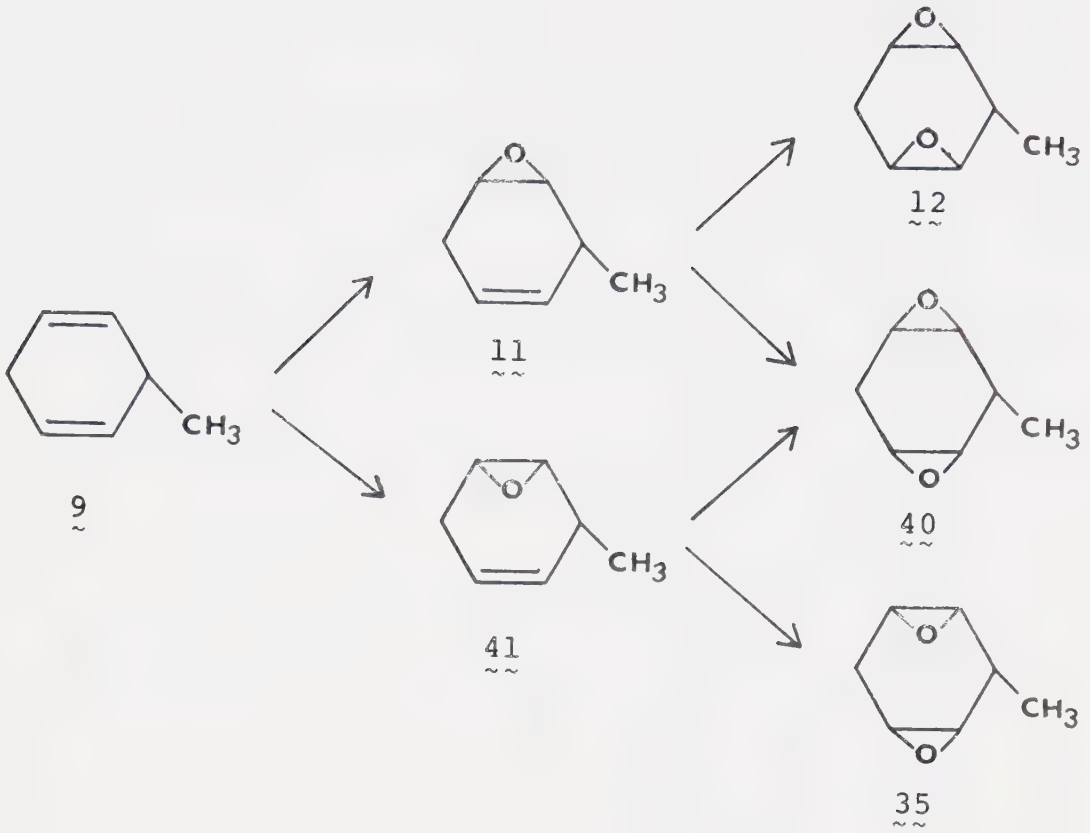
Evidently, should this occur in the case of the diepoxidation of 9 to yield 40, then the approach would not be useful. The first epoxidation on 9 could occur *trans* to the methyl group to afford 11, and the second epoxidation could also occur *trans* to the methyl group to afford 12 since the non-bonded interaction between an entering oxygen atom and a *cis*-methyl group could be prohibitive. The reaction pathway would depend on both a steric effect by methyl



group and an electrostatic effect by the first ring oxygen. Since there was no basis for a prediction, the direct diepoxidation on 9 with excess monoperphthalic acid was first examined. Tlc examination of the product showed the presence of three components--two major and one minor. The product was applied to a silica gel column for chromatographic separation into the three components. The pmr of the first major component (Fig. 3A) required it to be a mixture which contained monoepoxides of 9 as major products since the material showed a signal for olefinic hydrogen

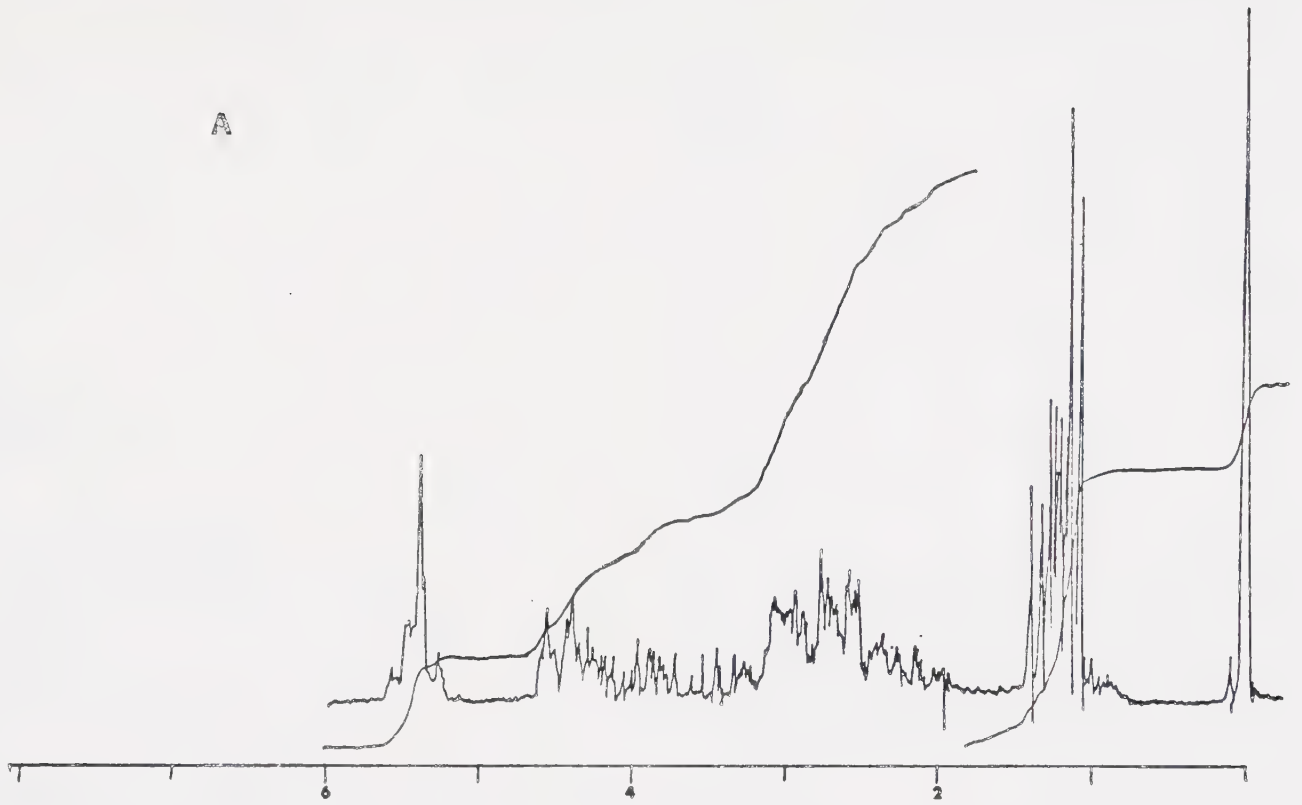


Fig. 3. 100 MHz pmr spectra for the three components of the direct epoxidation product on 3-methyl-1,4-cyclohexadiene (9) with monoperphthalic acid.

A. First fraction.

B. Second fraction.

C. Third fraction.

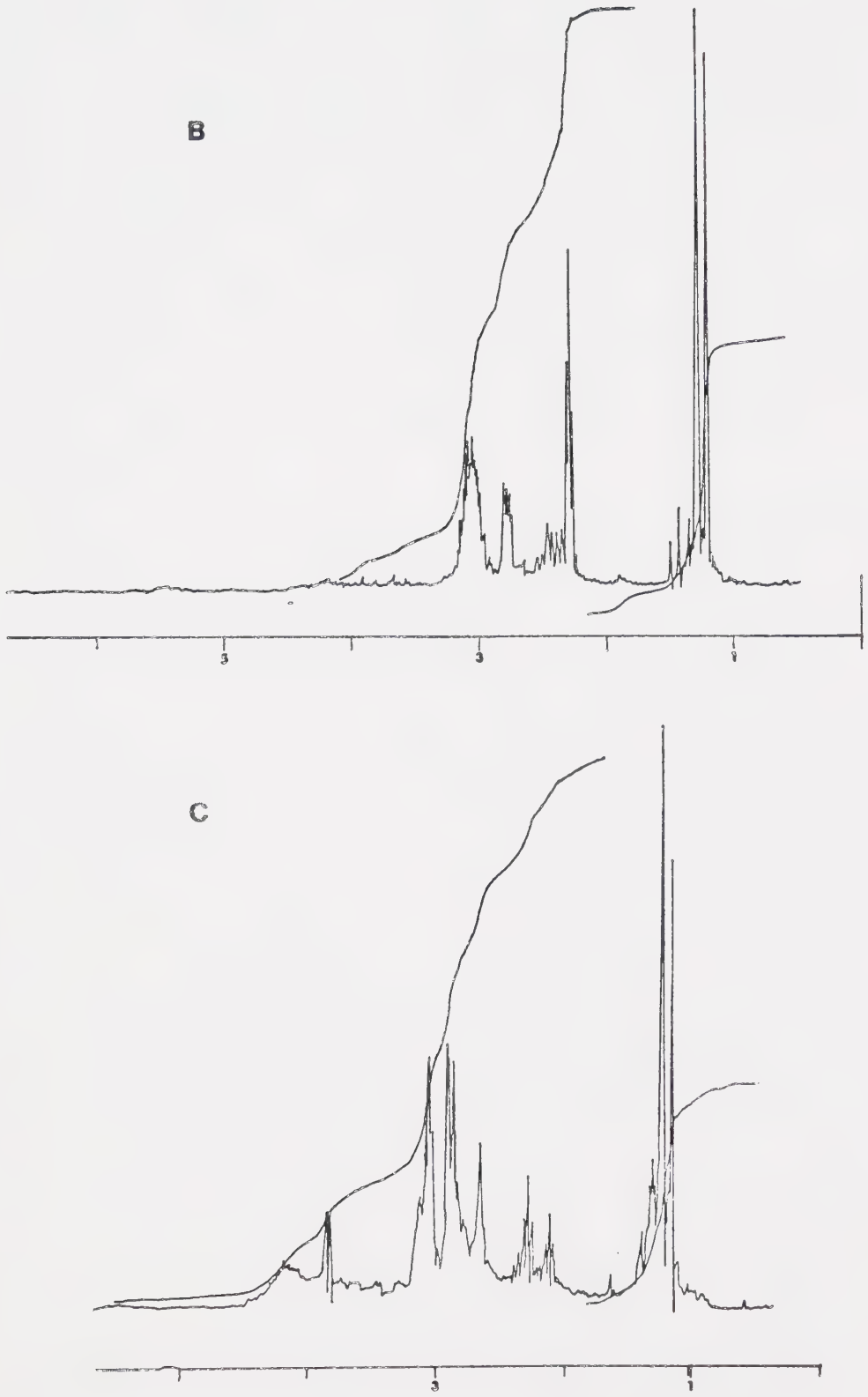


Fig. 3 (continued).

at about 5.4 ppm in addition to the multiplet in the epoxide ring proton region 2.6-3.1 ppm. The material appeared to be still a mixture since the olefinic hydrogen signal was present as a singlet superimposed on a broad quartet and four doublets of spacing 7 Hz were present in the region 1.0-1.4 ppm characteristic of *C*-methyl groups. Two minor doublets in the methyl proton region and multiplets in the region 3.2-4.6 ppm suggested the presence of unknown impurities. However, no attempt was made to further examine the mixture.

The second fraction was also major and the Rf value of the fraction on tlc was the same (0.50) as *trans*-diepoxide 10 of 1,4-cyclohexadiene. The spectrum, reproduced in Fig. 3B, showed this fraction to be still impure but, judging from the strong doublet of spacing 7 Hz centered at 1.12 ppm accompanied by a few small doublets, this fraction appeared to be a better than 80% pure sample of one of the three theoretically possible diepoxides. No signal for olefinic hydrogens around 5 ppm was observed and instead broad signals for four epoxide ring protons appeared in the region 2.9-3.1 ppm. By irradiation of the epoxide ring protons, a triplet centered at 2.27 ppm, a quartet at 2.40 ppm and doublet of doublets at 2.77 ppm were converted into a sharp singlet, a doublet and a broad singlet, respectively, indicating that these three protons are attached to the carbons adjacent to the epoxide rings. The hydrogen atoms which gave rise to the triplet and

quartet signals were not coupled since irradiation of one of the signals did not effect the other. Neither reliable assignment for these three proton signals nor the configurations of two epoxide rings were obtained even with spin decoupling technique since these signals are located too close to each other on pmr spectrum.

The third minor fraction, which showed similar Rf (0.33) to that of *cis*-diepoxide (7) of 1,4-cyclohexadiene, had the spectrum which is reproduced in Fig. 3C. It is seen that again impure substance was obtained but that the mixture consisted largely of one component. The spectrum was devoid of signals for olefinic protons and possessed signals in the general region expected for the epoxide ring protons of a 1,2:4,5-diepoxy-3-*C*-methylcyclohexane. Although upfield triplet of doublets centered at 2.28 ppm would be assigned to 6-position proton *trans* to the epoxide oxygens in *cis*-configuration, again, this pmr did not allow conclusions as to the configurations of this diepoxide.

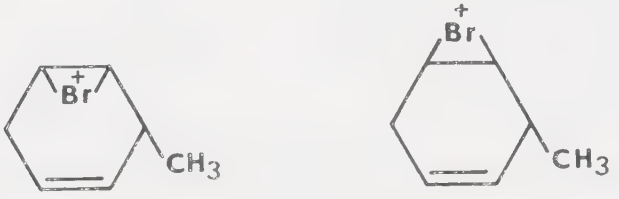
This direct diepoxidation approach to the preparation of *trans,trans*-1,2;4,5-diepoxy-3-*C*-methylcyclohexane (12) would be useful if the major second fraction were the desired product. However, this major fraction proved to be the *cis,trans*-diepoxide 40 (see page 61).

Since the yields obtained in the direct epoxidation were not attractive, it was decided to examine indirect epoxidation. It has been reported (17) as shown below that treatment of 1,4-cyclohexadiene with N-bromosuccinimide

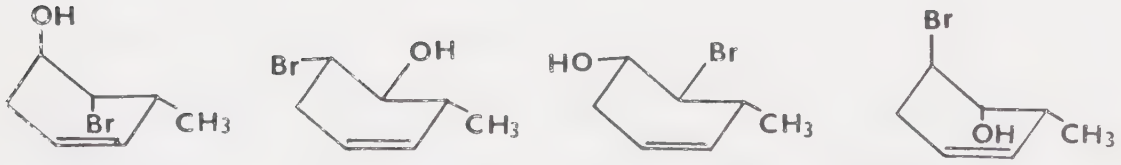
(NBS) in water produced the monobromohydrin 42 which, by the epoxidation with monoperphthalic acid, was converted mainly to the *cis*-epoxy-alcohol 6, which in turn was treated with aqueous sodium hydroxide to afford *cis*-diepoxide 7.

42 6 7

The hydroxybromination of 9 is expected to proceed by way of either the *cis*- or *trans*-bromonium ions 43 and 44. Steric considerations are expected to favor the formation of the *trans*-isomer 44 which should yield the bromohydrins 16 and 17 (32).



43 44



14 15 16 17

If the formation of the bromonium intermediates is an irreversible step, as it is assumed for the bromination in nonpolar solvents, the steric course of the addition will be controlled first by the relative rates of electrophilic attack on the two faces of the carbon-carbon double bond and then by those of the two alternative modes of nucleophilic opening of the bromonium ions (33). In this case, the bromohydrin isomer 16 derived from *trans*-bromonium ion 44 would be the major component in the reaction product (Fig. 4).

On the other hand, if the bromonium ions were formed in a reversible prerate-determining step which is easily realized by the hydroxybromination in hydroxylic solvents, the steric course of the addition could be controlled mainly by the difference in the transition-state free energies of the nucleophilic steps, provided that they are sufficiently slower than the formation of the bromonium ions and their reversal to the alkene. Supposing that the hydroxybromination reaction on 9 is performed in hydroxylic solvents, since path D is less favored than path C, the *trans*-bromonium ion 44 would revert in part to the starting olefin 9 and the reaction would proceed predominantly *via* the *cis*-ion 43 to give an excess of the bromohydrin 14 (18) since the attack of hydroxyl anion on the 1-position of *cis*-bromonium ion 43 is easier than on the 2-position.

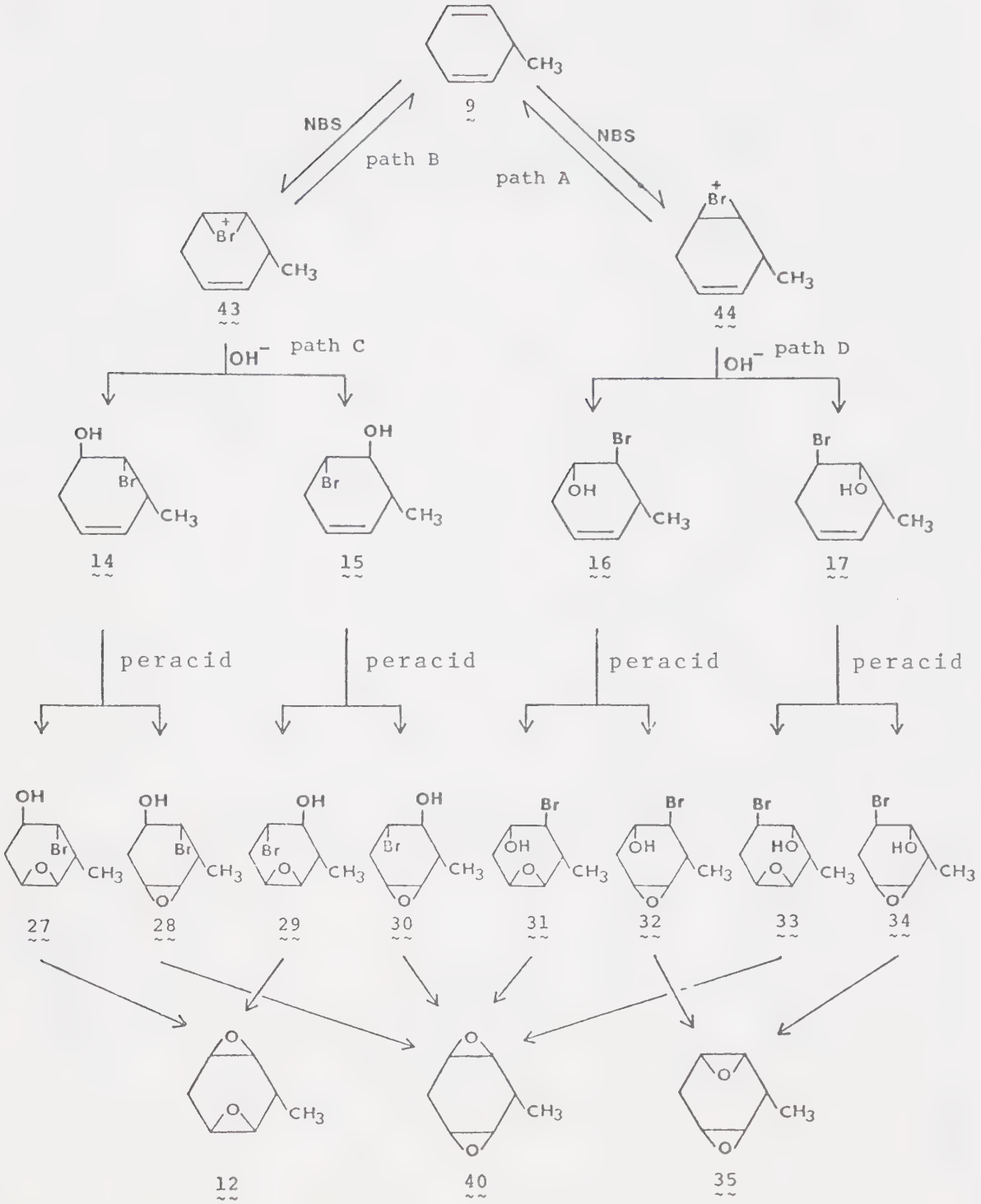


Fig. 4. Preparation of diepoxides of 3-methyl-1,4-cyclohexadiene *via* indirect epoxidation.

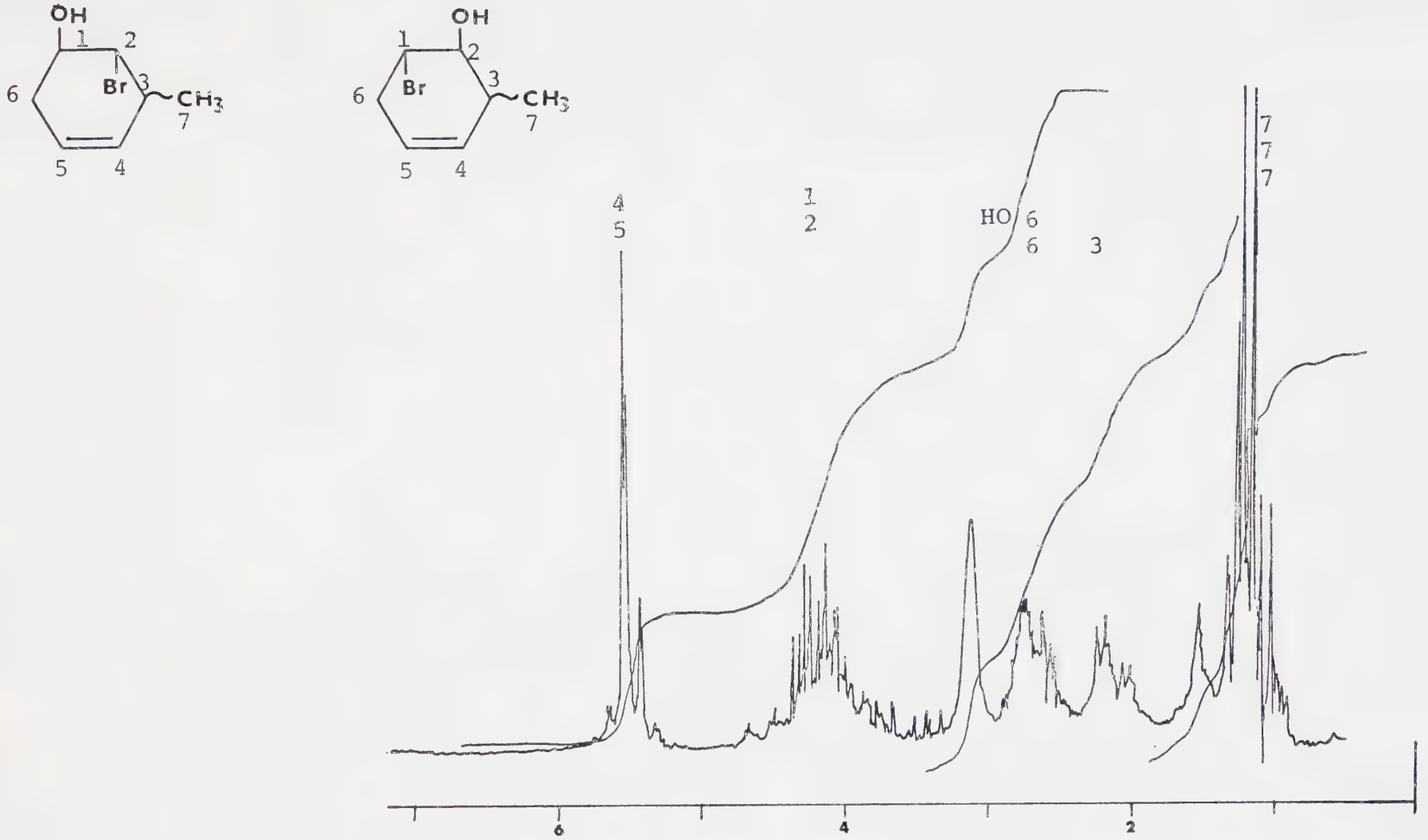
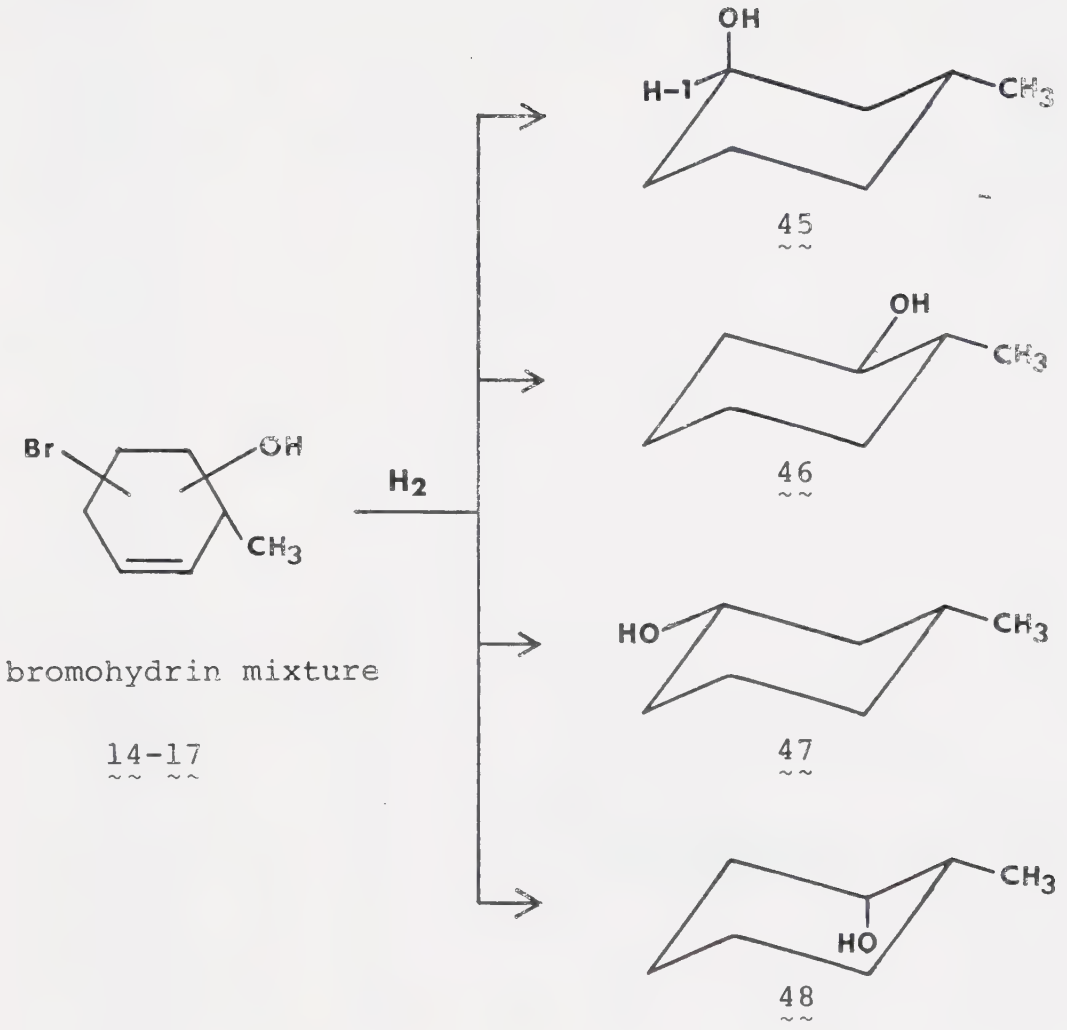


Fig. 5. 100 MHz pmr spectrum for the mixture of bromohydrin derivatives of 3-methyl-1,4-cyclohexadiene. The assignments are based on the above numbering for the various carbon atoms.

As can be appreciated from the reaction scheme presented in Fig. 4, the desired diepoxide 12 can be prepared only *via* the *cis*-bromonium ion 43. Therefore, hydroxylic solvent had to be chosen for the hydroxybromination reaction on 9. Upon treatment of 9 with an equimolar quantity of NBS in aqueous dioxane, a mixture of the monobromohydrin derivatives 14-17 was obtained in 97.2% yield. Although the formation of the four possible isomers was indicated by both thin layer chromatography and pmr spectrum (Fig. 5), no attempt was made to separate the components. However, the following experiment was done to check the presence of bromohydrin derivative 14 as the major component in the reaction mixture.

It could be anticipated that the hydrogenation-hydrogenolysis of the crude mixture of bromohydrins (14-17) would yield a mixture of methylcyclohexanols (45-48), the composition of which would reflect the composition of the mixture of bromohydrins. Therefore, the mixture was reacted with hydrogen (26) in the presence of palladium and triethylamine using methanol as solvent. The product was bromine free and the pmr spectrum (Fig. 6) was devoid of signals for olefinic protons.



bromohydrin mixture

14-17

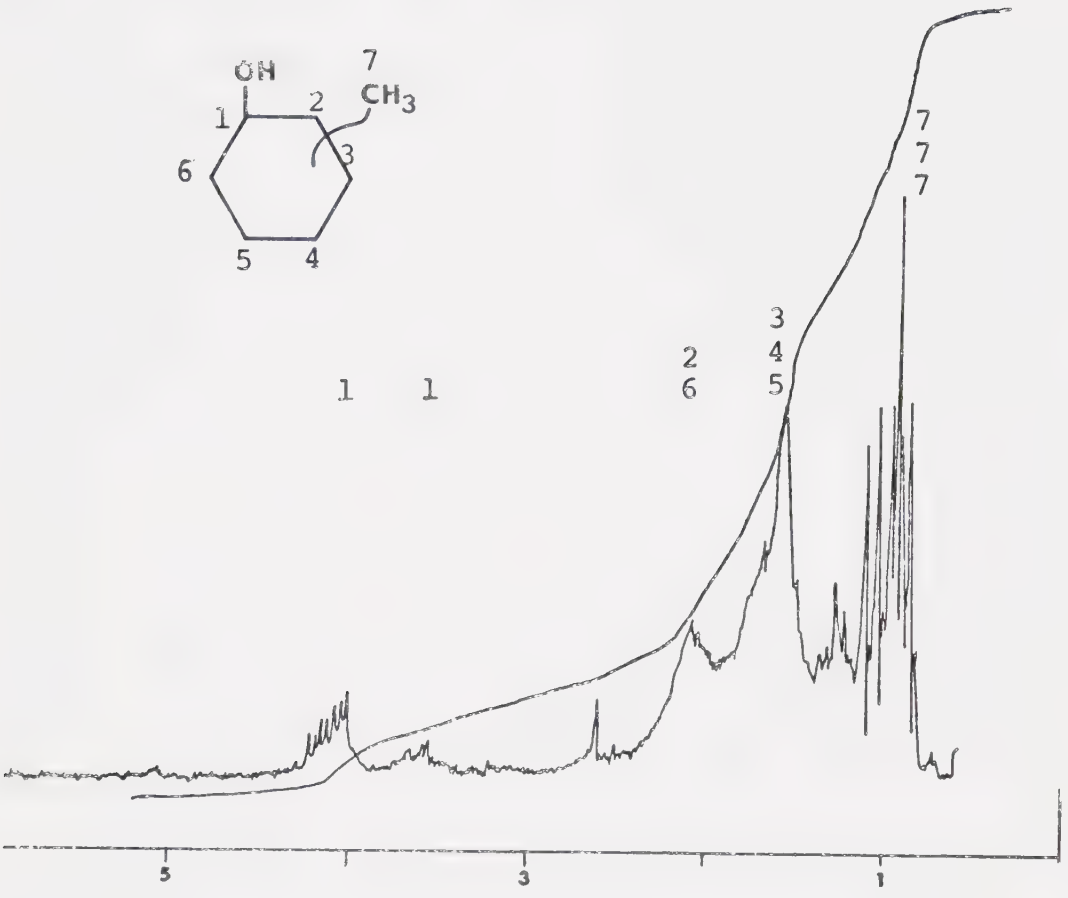


Fig. 6. 100 MHz pmr spectrum for the mixture of the hydrogenated products derived from bromohydrin derivatives. The assignments are based on the above-shown numbering for the various carbons.

Furthermore, signals occurred in the region 3.4-4.1 ppm of total relative intensity of near one and these chemical shifts correspond well for the chemical shifts reported (34) for H-1 of the isomeric monomethylcyclohexanols. The signal for H-1 of 45 is to lower field than are those of H-1 for the isomers 46, 47, and 48 (34) as expected because the compound must have H-1 largely in equatorial orientation and not shielded by a neighboring methyl group. In fact, the main intensity for H-1 type signal was at 3.96 ppm suggesting that the main component was 45. This was confirmed by cmr spectroscopy. As seen in Table 1, Roberts and coworkers (35) have reported the chemical shifts for the four isomeric methylcyclohexanols (45-48) and the major signals in the cmr spectrum for the mixture, as seen in Fig. 7 and Table 1, are in excellent correspondence with the spectrum for 45.

Therefore, it could be concluded that the main component in the mixture of bromohydrins was the *arabino*-isomer 14. Judging from the pmr spectrum of the mixture of methylcyclohexanols (Fig. 6), the mixture could be expected to also contain a substantial amount of either or both 47 and 48 but very little of 46. With this information on the composition of the mixture of bromohydrins it could be anticipated that the preparation could in fact be used to prepare the desired 5-*C*-methyl-2,5-dideoxystreptamine (5). That is, epoxidation of 14 could be expected to be not only *trans* to the bromine as found for the epoxidation of

Table 1. Pmr and Cmr Chemical Shifts of Methylcyclohexanols in CCl_4

Compound	H-1 Chemical Shifts	C-13 Chemical Shifts						
	H-1	C-1	C-2	C-3	C-4	C-5	C-6	Methyl-C
45	3.96	66.8	41.5	26.9	34.7	20.5	33.1	20.5
46	2.98	76.9	40.0	34.3	26.1	25.7	35.4	19.1
47	3.45	70.8	44.3	32.0	35.1	24.7	44.3	22.8
48	3.75	71.4	36.1	29.6	24.5	21.8	32.1	16.5
main signals of mixture	4.00 (65%) 3.40-3.80 (35%)	66.9	41.6	26.6	34.2	22.0	33.2	20.0

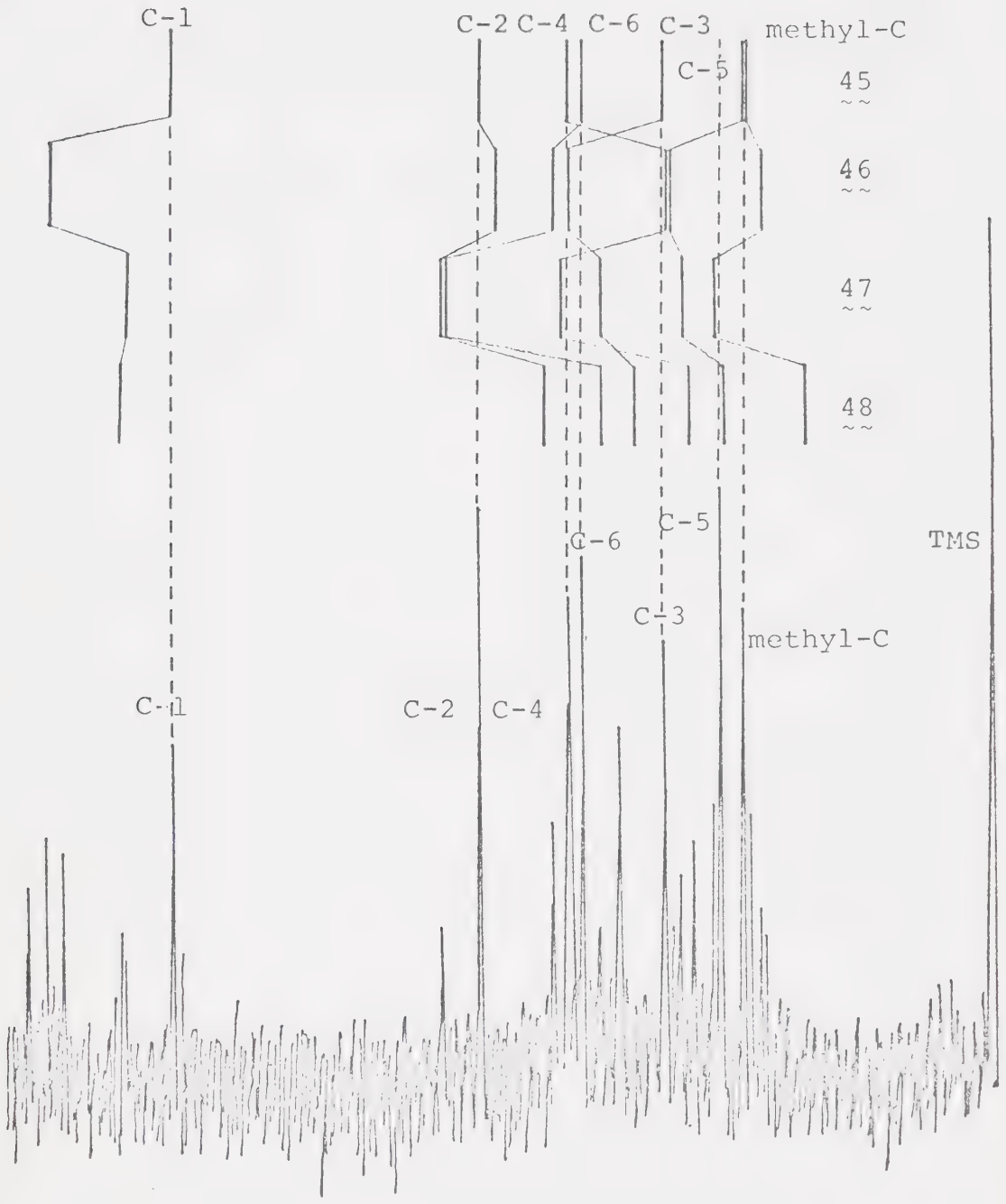


Fig. 7. The ^{13}C chemical shifts of the four isomeric methyl cyclohexanols (45 to 48, see Table 1) and the 22.6 MHz cmr spectrum for the mixture obtained on hydrogenating the mixture of bromohydrins 14 to 17. It is seen that the signals for 45 correspond well with the most intense signals in the spectrum of the hydrogenated product.

the monobromohydrin of 1,4-cyclohexadiene by Craig *et al* (17) but also *trans* to the methyl group as found and discussed above for the epoxidation of 3-methyl-1,4-cyclohexadiene.

At this point, the option existed of trying to separate the mixture of the bromohydrins to achieve pure samples of the components. However, it was decided to convert the mixture to the diepoxides in the anticipation that these compounds could be separated and thereby allow a more efficient overall preparation. It turned out to be not readily possible to separate the diepoxides but time did not allow a repetition of the preparation of the mixture of bromohydrins. As will be seen later on, not only was the separation of the diepoxide very difficult but also the separation of the isomeric 5-methyl-2,5-dideoxystreptamines formed from those compounds. Certainly, future preparations should involve effort to separate the bromohydrins as such or as derivatives.

The mixture of bromohydrin derivatives 14-17 (main isomer is 14) was treated with an excess of monoperphthalic acid in diethyl ether. An 89% yield of a product which could be resolved into two bands by tlc was obtained. It is reported that in the epoxidation of hydroxycyclohexene derivatives, the *cis*-epoxy alcohols are the major isomers owing to hydrogen bonding of the peracid with the hydroxy group (17,36). It is to be expected, therefore, that four possible *cis*-epoxy alcohols 27, 29, 32, and 34 would be

derived from the four bromohydrins (14-17), respectively. However, epoxidation *trans* to the hydroxyl group could be expected for the bromohydrin 17 which possesses the methyl group *cis* to the hydroxyl. Thus, the methyl group could conceivably direct the epoxidation to form 33 rather than 34 (p. 38). Since the bromohydrin 14 was the main component of the mixture, it was expected that 27 would be a major product. The product was found on examination by cmr (Fig 8) to contain five main components, since the cmr spectrum of the product showed five signals in the *C*-methyl region 16.9-20.15 ppm.

The mixture of epoxy bromohydrins was, without separation, treated with *N* aqueous sodium hydroxide to afford diepoxides; a mixture comprising structures 12, 35, and 40 being expected. The reaction provided an oily product in near quantitative yield based on a simple dehydrobromination reaction.

Examination by tlc showed the presence of at least two major components. Column chromatography on silica gel separated these substances into the two fractions. The first fraction to elute was obtained in 19% yield and possessed the pmr spectrum reported in Fig. 9B. It is seen that this fraction is highly enriched (~80% relative to the intensity of the methyl group signals) in one compound which, because of the chemical shifts of the hydrogens in the region 2.7-3.1 ppm, required it to be a diepoxide (for example, the diepoxide 7 has the spectrum displayed

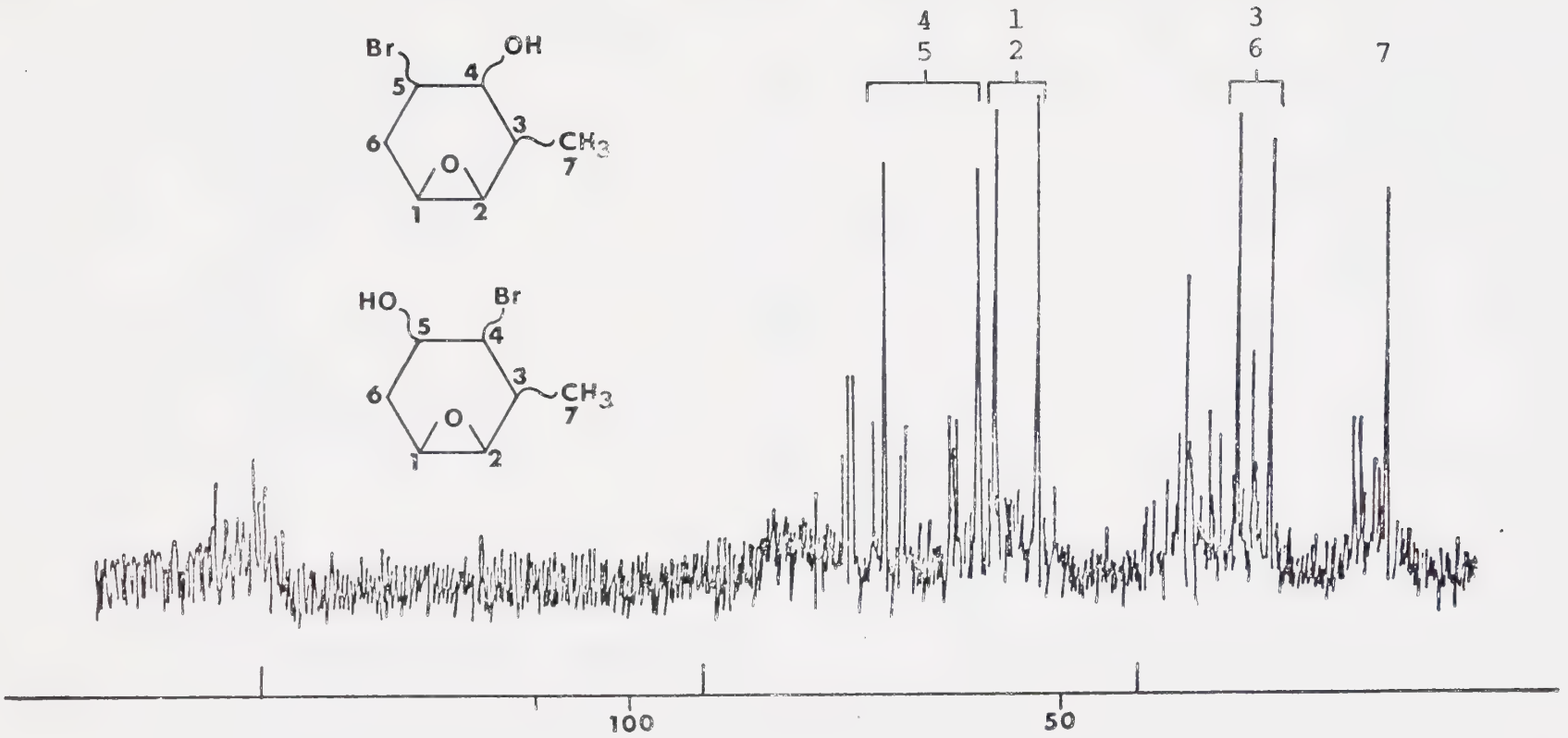


Fig. 8. 22.6 MHz cmr spectrum for the mixture of epoxy bromohydrin derivatives of 3-methyl-1,4-cyclohexadiene. The assignments are based on the above-shown numbering for the various carbon atoms.

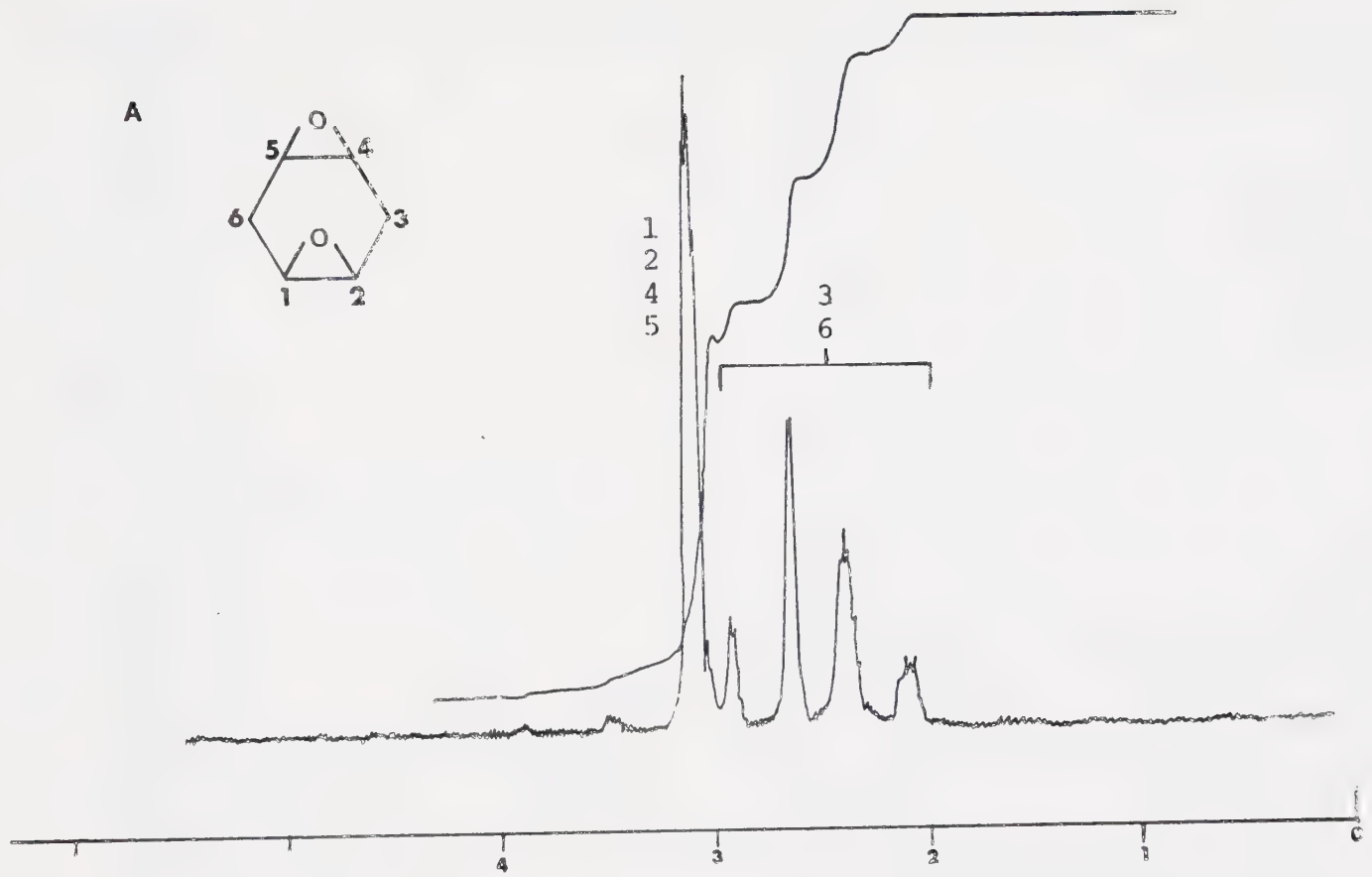


Fig. 9. Nmr spectra for diepoxides. The assignments are based on the numbering for the various carbon atoms.

A. 60 MHz pmr spectrum of *cis*-diepoxide (7) of 1,4-cyclohexadiene.
B. 100 MHz pmr spectrum for *cis, trans*-1,2;4,5-diepoxy-3-*C*-methylcyclohexane (40).
C. 25.2 MHz cmr spectrum for (40).

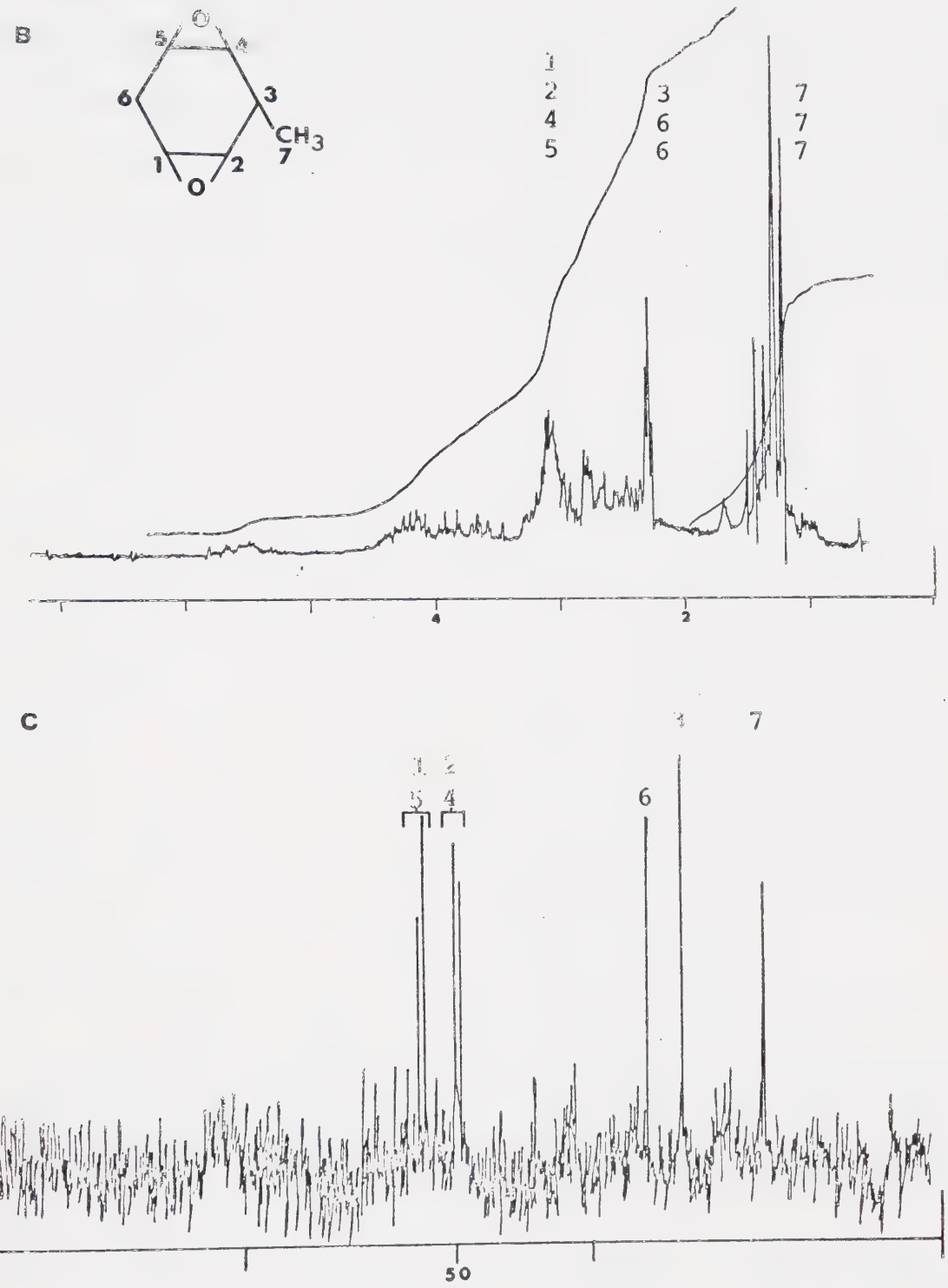


Fig. 9 (continued)

in Fig. 9A, where it is seen that four equivalent hydrogens in the epoxide groups give their signal at 3.09 ppm). The cmr spectrum (Fig. 9C) shows a spectrum consistent with the dissymmetric structure for the *cis,trans*-diepoxide 40. This follows from the equal intensities found for the ring carbons. On this basis, it may be concluded that compounds 28, 30, 31, and/or 33 were not major components in the product obtained on the epoxidation of the mixture of bromohydrins 14-17 (Fig. 4).

The second fraction (80% yield), although it gave only one spot on tlc, was a mixture in view of the two $C-CH_3$ group signals present in its pmr spectrum (Fig. 10A). The relative intensities of these signals required an about 6:1 mixture. The chemical shifts of the ring protons required it to be a mixture of diepoxides. The cmr spectrum of the mixture (Fig. 10B) clearly required that the main product (and likely the minor product) has the two epoxide groups in *cis*-relationship. This follows from the 2:2:1:1 relative intensities found for the ring carbons.

As will be seen later on, the major compound was the *trans,trans*-diepoxide 12 and, indeed, the minor compound was the *cis,cis*-diepoxide 35. Therefore, the main product formed by way of the epoxidation of the mixture of bromohydrins was 27; that is, the main course of the epoxidation involved insertion of the oxygen *trans* to the vicinal methyl group, a course of reaction in good accord with

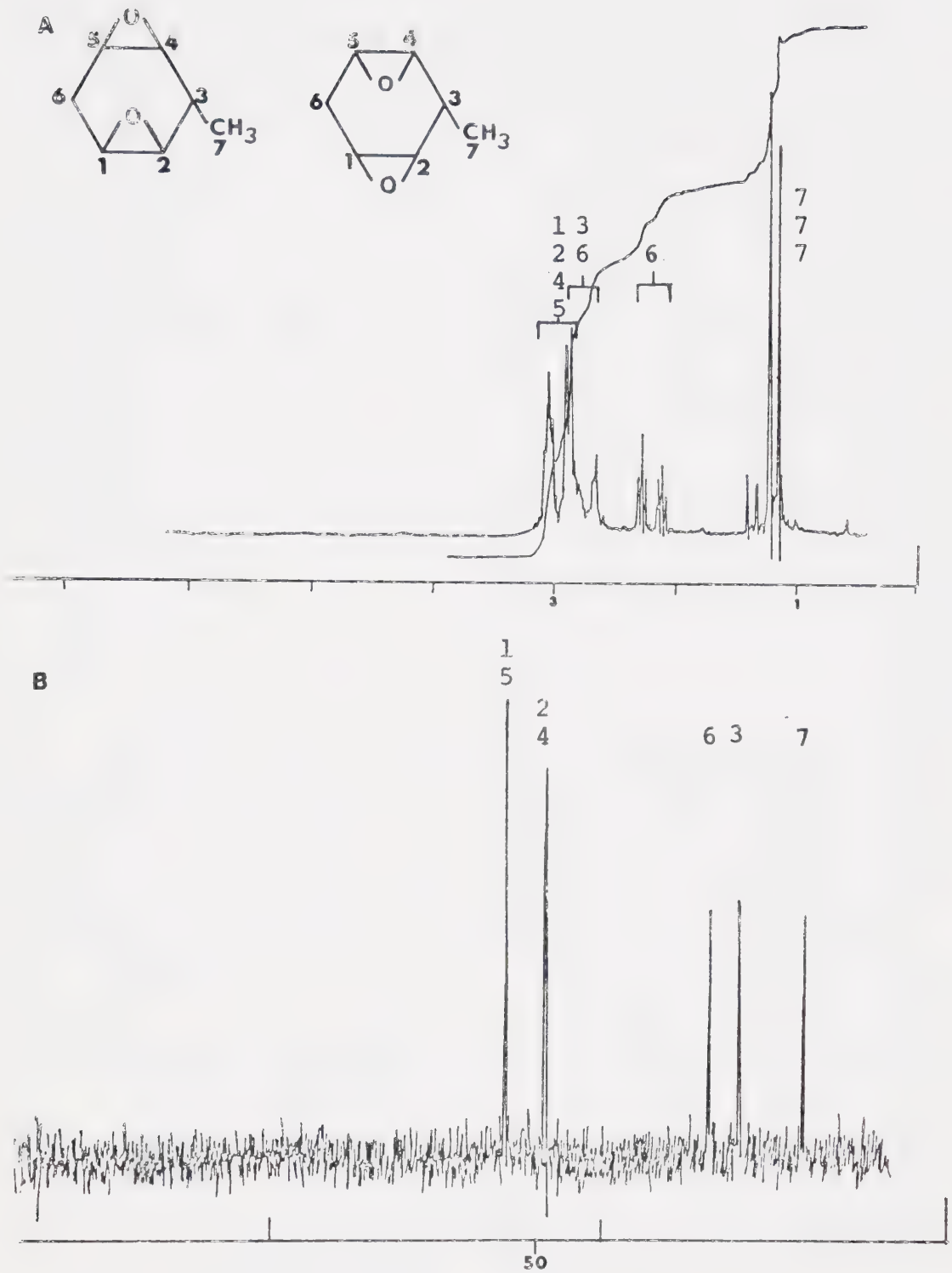


Fig. 10. Nmr spectra for the mixture of *cis*-diepoxides of 3-methyl-1,4-cyclohexadiene. The assignments are based on the above numbering for the various carbon atoms.

A. 100 MHz pmr spectrum in $CDCl_3$.

B. 22.6 MHz cmr spectrum in $CDCl_3$.

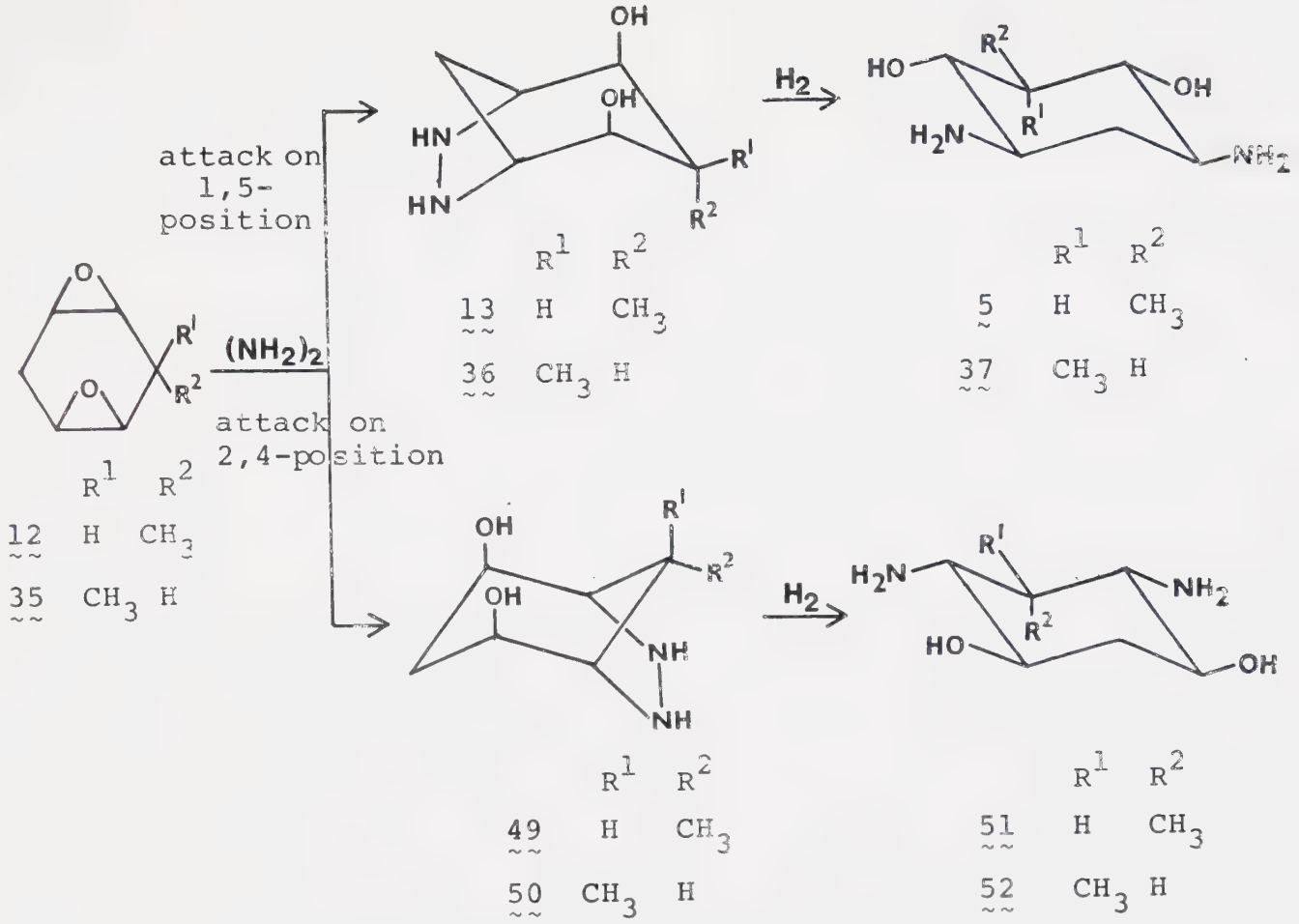


Fig. 11. Preparation of 2,5-dideoxy-methylstreptamine derivatives from *cis*-diepoxides.

steric considerations. The formation of 35 required the presence of one or both of 32 and 34 in the mixture of monoepoxybromohydrins (Fig. 4).

Treatment of the mixture of symmetric diepoxides (second fraction) with anhydrous hydrazine in refluxing 2-methoxyethanol following the method by Suami *et al* (16) afforded, in 50% yield, off-white crystals which readily decomposed within a few hours after separation. This instability may be the reason why the yield was as low as 50% and no attempt to analyze the product was made. The reaction scheme is presented in Fig. 11. In the case of compound 7, which has no methyl group on the cyclohexane ring, an 85% of yield of 8 was realized (16).

The crude product was treated with hydrogen in the presence of platinum catalyst in a Parr Pressure Reaction Apparatus. By this reaction, a basic crystalline mass was isolated as a hydrochloride salt in 61% yield. It was found by inspection of the pmr spectrum (Fig. 12A) for this product that it was a mixture of two components in an about a 4:1 ratio as judged from the relative intensities of doublet signals occurring at 1.32 and 1.12 ppm with spacings of 7.6 Hz. The cmr of this mixture, reproduced in Fig. 12B, required it to contain either 5-equatorial-methyl-2,5-dideoxystreptamine (5) or 2-axial-methyl-2,5-dideoxystreptamine (51) as the main product because of the 2:2:1:1 relative intensities found for the ring carbons. The

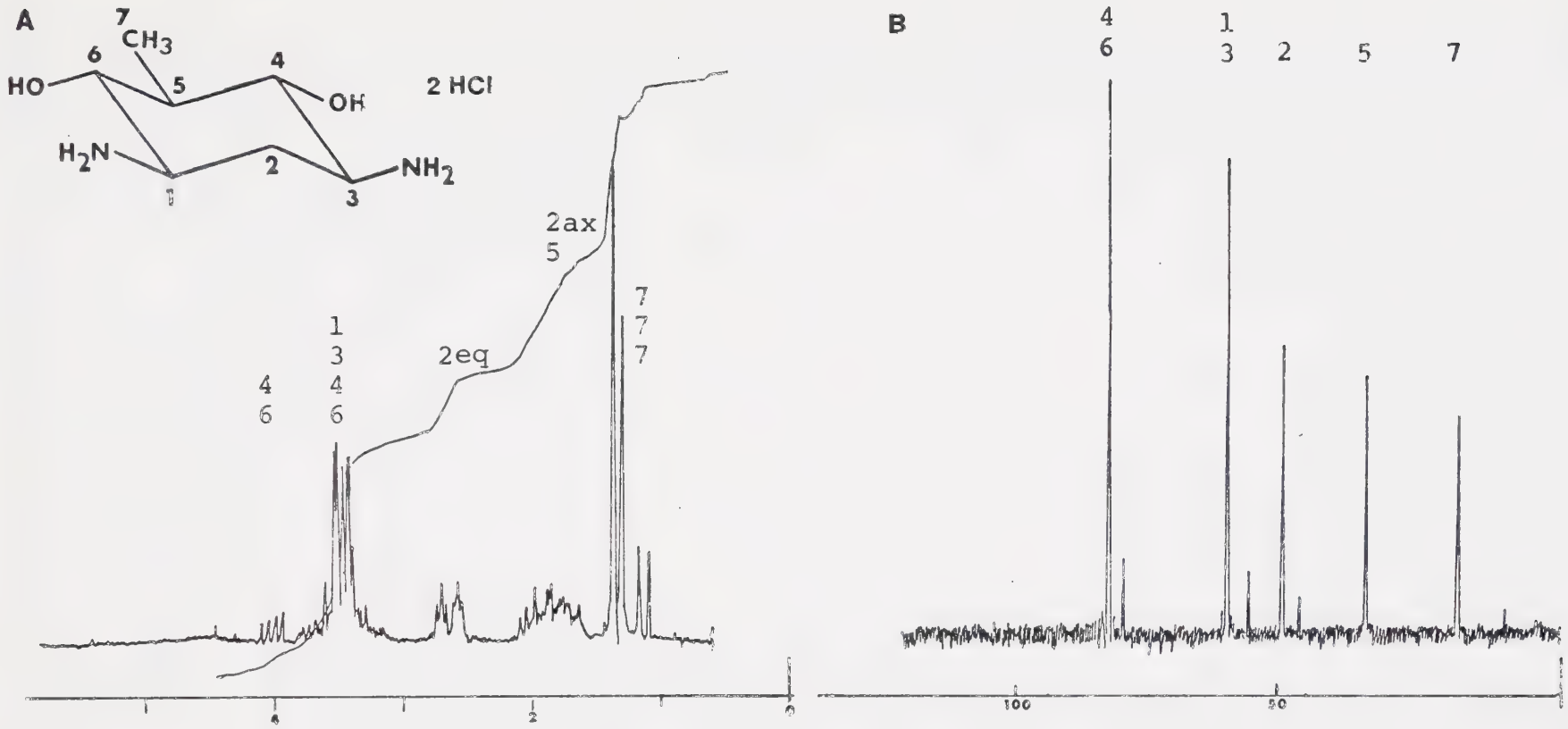


Fig. 12. Nmr spectra of crude 2,5-dideoxy-5-*C*-methylstreptamine dihydrochloride. The assignments are based on the above numbering for the various carbon atoms.

A. Pmr spectrum in D_2O.

B. Cmr spectrum in D_2O.

minor product would be either 5-axial-methyl-2,5-dideoxystreptamine (37) or 2-equatorial-methyl-2,5-dideoxystreptamine (52). When the hydrazine attacked the mixture of *cis*-diepoxides, 12 and 35, there were two possible points of attack either at the 1- and 5-positions or at the 2- and 4-positions as shown in Fig. 11. The observation that the more intense doublet for the methyl protons of the pmr spectrum was at lower field (δ 1.32) than that of the other doublet (δ 1.12) seemed to require that the methyl group in the main product be in equatorial orientation. Integration indicated the presence in the main component of two protons which gave signals in the range 1.62-2.10 ppm and one proton at 2.63 ppm. Presumably, the upfield signals arose from two axial hydrogens at C-2 and C-5 and the lower field signal from the equatorial H-2. However, the pmr spectrum did not allow reliable assignments of structure and configuration and, therefore, the diphthalimido diacetate derivative of the mixture was prepared. A near 100% yield of a crystalline product possessing the correct analysis for diphthalimido-diacetoxy-methylcyclohexane was obtained. The product was purified by column chromatography. Although the recrystallized product from methanol showed a melting point in the range of 292-294°C, the pmr spectrum (reproduced in Fig. 13) indicated an 85:15 mixture. The more intense doublet for methyl protons (which was at lower field than the weaker doublet in the mixture of dideoxymethylstreptamine) appeared to higher

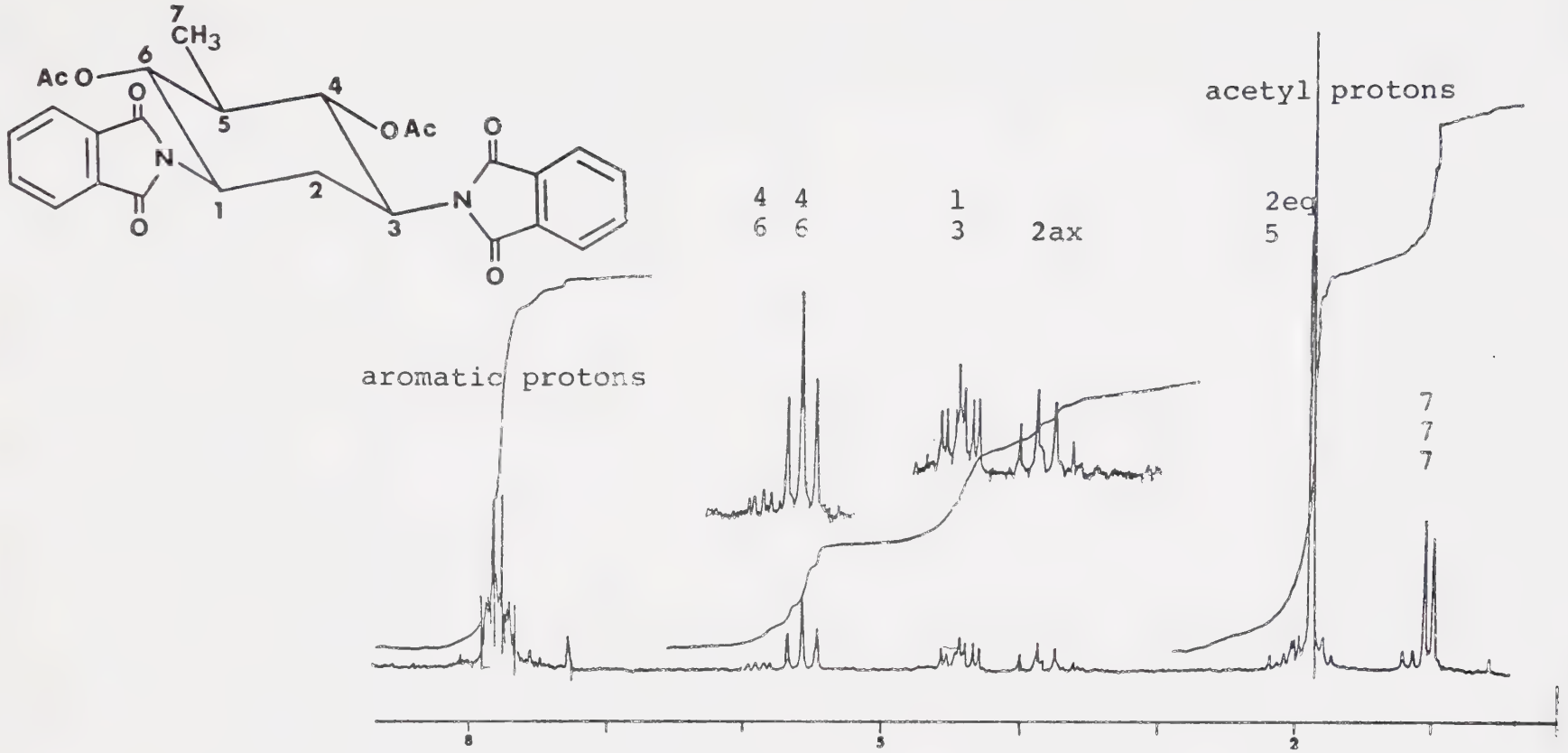


Fig. 13. 100 MHz pmr spectrum of crude (1,3,5/4,6) 1,3-bisphthalimido-5-*C*-methyl-4,6-cyclohexanediol diacetate (38). The assignments are based on the above numbering for the various carbon atoms.

field. Thus, it could be presumed that because of the shielding effect of the ring currents of the two phthalimido groups, the methyl group of the main isomer was on the same side of the cyclohexane ring as were the phthalimido groups. Six acetyl protons and eight aromatic ring protons were present at 1.87 and 7.80 ppm, respectively.

It is known that a proton which is attached to a carbon bonded to an acetoxy group in a cyclohexane derivative appears to lower field than a proton attached to a carbon bonded to a phthalimido group on the same ring. Therefore, a triplet in the lower field (δ 5.57) could be assigned to the equivalent H-4 and H-6 atoms (the numbering is displayed in Fig. 13). Because of the magnitude of the coupling (~11 Hz), the triplet requires the H-4 and H-6 to be coupled about equally with two vicinal protons which are also in axial orientation. In view of its chemical shift, the octet at 4.27-4.58 ppm with spacings of 4, 10.5 and 12 Hz is assigned to H-1 and H-3 which are attached to carbons bonded to phthalimido groups. This signal is in accord with the structure assigned to the major component and presented in Fig. 13 since the magnetically equivalent axial hydrogens for H-1 and H-3 would each be strongly coupled with two vicinal axial hydrogens and more weakly coupled with a vicinal equatorial hydrogen. The quartet at 3.8 ppm has three spacings of 12 Hz. This signal would be in accord with that of an axial hydrogen coupled (12 Hz)

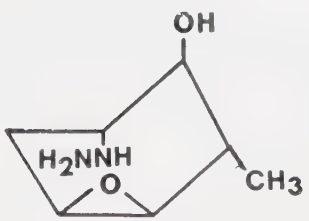
with two equivalent and vicinal axial hydrogens and further coupled with a geminal hydrogen by 12 Hz. These coupling constants would be in good accord with expectation for the axial hydrogen at position 2 for the structure depicted in Fig. 13. Presumably, the two vicinal phthalimido groups strongly shield the equatorial H-2 hydrogen so that its signal is shifted to the 2 ppm region near the signals for the acetoxy groups. Indeed, integration required the presence of two ring hydrogens along with the signals for the acetyl groups at this position in the spectrum. That one of these hydrogens is the methine H-5 was established by spin decoupling. Thus, irradiation of the more intense signal for *C*-methyl group at 1.04 ppm caused the collapse of the multiplet near 2 ppm and the irradiation of the multiplet at 2 ppm in reverse converted the doublet for the methyl group into a singlet. The irradiation of the multiplet at 2 ppm converted the triplet at 5.57 ppm into broad doublet indicating the methine proton is adjacent to the acetoxy group.

The small quartet at 5.87 ppm with spacings of 5 and 11 Hz could be assigned to H-4 and H-6 of the minor component which has the *C*-methyl group in axial orientation. These protons would be expected to occur to lower field than those of the equatorial methyl isomer because of the decreased shielding effect of the axial *C*-methyl group. Furthermore, each of these protons would be coupled with an axial proton (H-1 or H-3) and an equatorial

proton (H-5) in accord with the observed coupling constants.

Therefore, it could be concluded that the major product in the dideoxymethylstreptamine mixture was the desired equatorial-methyl isomer 5 and the minor one the axial-methyl isomer 37.

Finally, in view of the structure for compound 5, it could be concluded that it arose from the *trans,trans*-diepoxide 12. Therefore, 12 was the main component of the product obtained by way of the indirect diepoxidation of the 3-methyl-1,4-cyclohexadiene. Furthermore, the formation of 5 from 12 requires that the attack of 12 by hydrazine occurred predominantly at one of the chemically equivalent 1- and 5-positions. Consequently, following the Fürst-Plattner rule for the opening of epoxide rings, the first product of the attack of diepoxide 12 was 53



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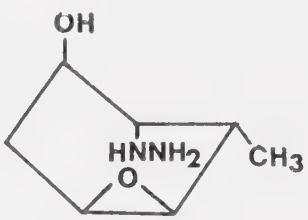

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which has the methyl group in *quasi*-axial orientation and not 54. Thus, hindrance by the methyl group to attack by hydrazine at the 2- or 4-positions of 12 appears to set the stereochemical course of the reaction.

The pmr spectrum of crude 12 (Fig. 10A) is seen to be very different from that (Fig. 3B) of the major product of the direct diepoxidation of 3-methyl-1,4-cyclohexadiene but very similar to that (Fig. 3C) of the minor component. On the other hand, the pmr spectrum (Fig. 9B) of crude *cis,trans*-diepoxide 40 is very similar to that (Fig. 3B) of the major diepoxide formed directly from the diene. Therefore, there can be no doubt that the direct diepoxidation of 3-methyl-1,4-cyclohexadiene provides mainly the product which has the oxide rings in *trans*-relationship as is the case for the epoxidation of 1,4-cyclohexadiene (17).

Several techniques were employed in attempts to separate 5 from 37. The first attempt was by recrystallization from water-ethanol-ether mixed solvent. The percentage content of axial methyl isomer in each crop was determined by comparison of the signals for methyl groups in the pmr spectra of each crop. The content of 37 was found to be 26% (third crop), 21% (second crop) and 16% (first crop), respectively, as shown in Fig. 14A, B, C. The solvent system was then used for fractional recrystallization (28) starting from 1.2 g of crude mixture. The results are summarized in Fig. 15. After seven recrystallizations, 110 mg of needles were obtained. However, it was found that the material still contained 14% (11% by the inspection on cmr spectrum) of 37 as shown in Fig. 14D.

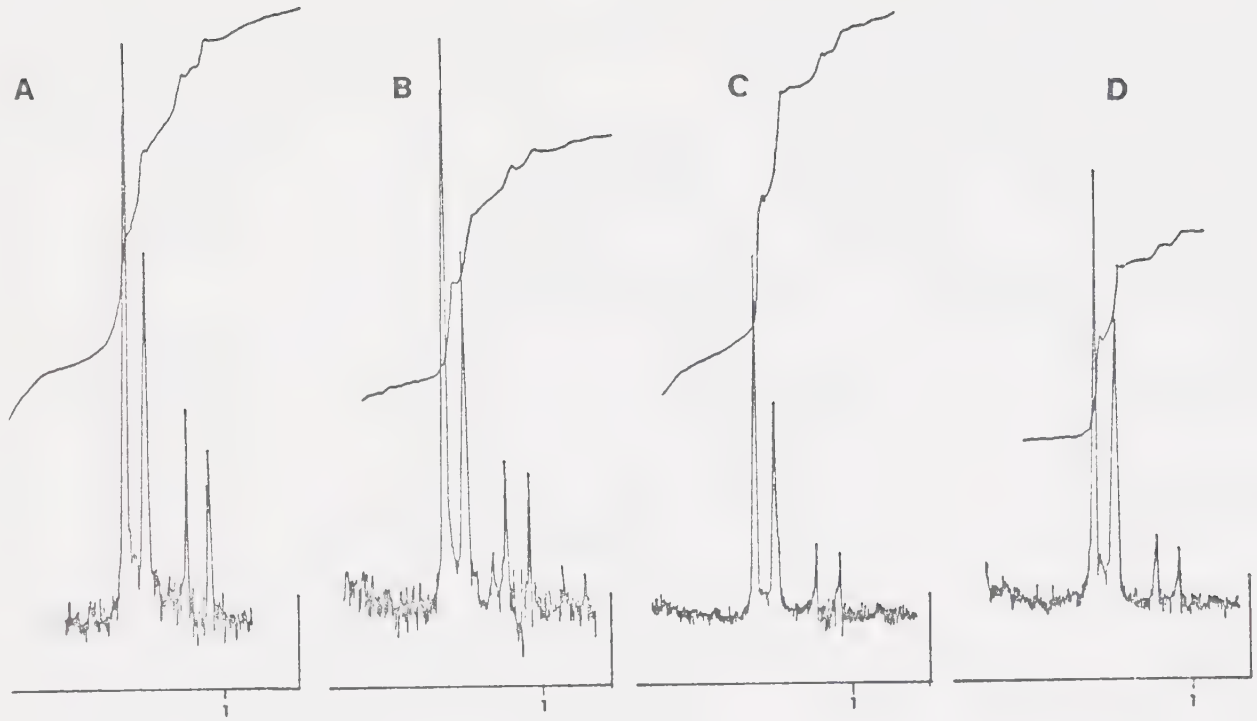

Fig. 14. Methyl proton region of pmr spectra for each crop of 2,5-dideoxy-5-*C*-methylstreptamine dihydrochloride.

A. Third crop of recrystallization.
B. Second crop of recrystallization.
C. First crop of recrystallization.
D. Solid-7 of the fractional recrystallization summarized in Fig. 15.

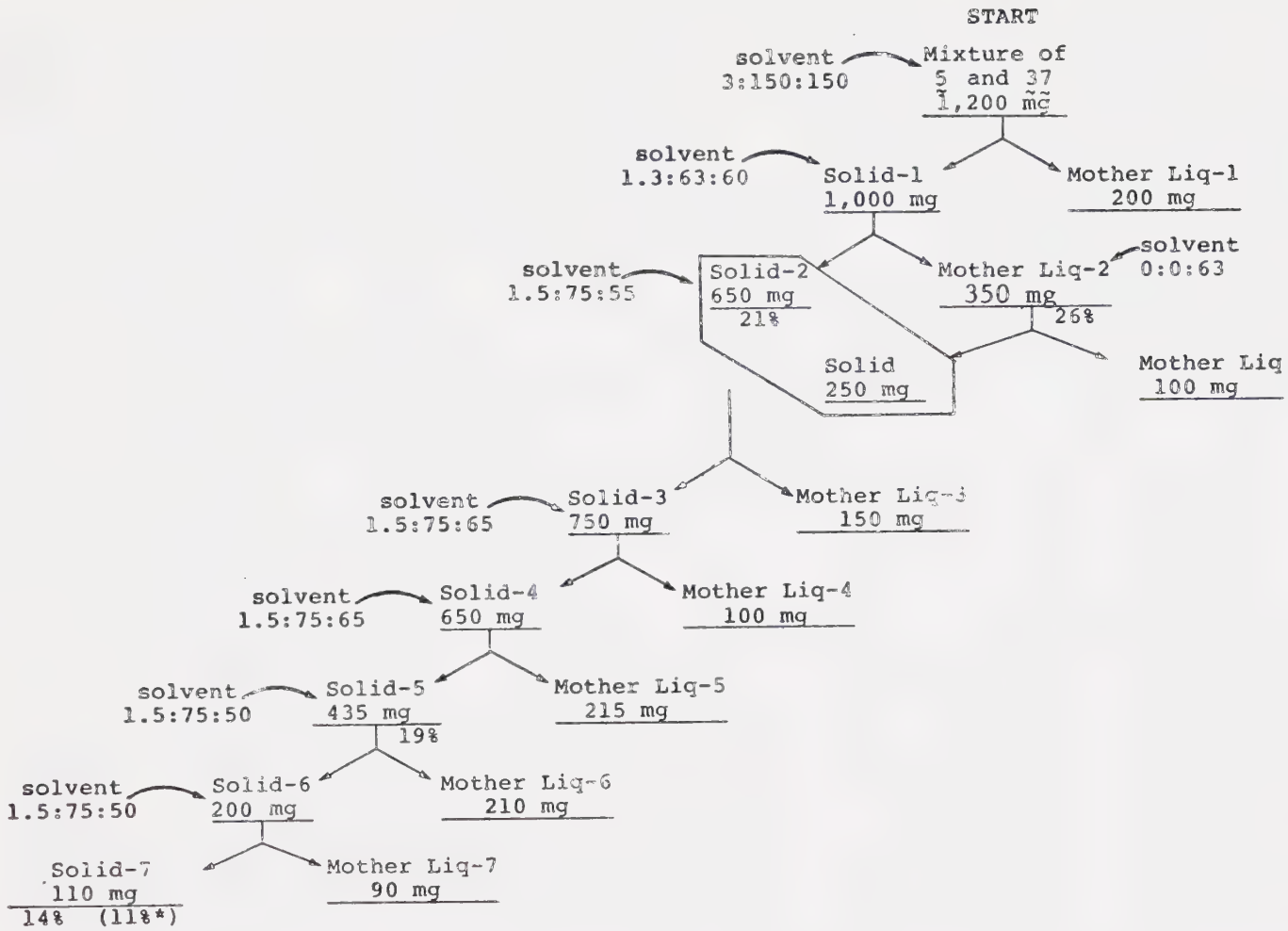

Fig. 15. Summary of the fractional recrystallization. Solvent system employed is shown as the volume (ml) of water:ethanol:ether. Values of percentage show the percentage content of isomer 37 in each crop.

*By the comparison of areas of C-2 signals for each isomer on cmr spectrum.

Descending paper chromatography was performed using 1-butanol:pyridine:water:acetic acid = 6:4:3:1 mixture as developing solvent. The Rf value obtained was 0.22 for both isomers 5 and 37.

An attempt to chromatograph the mixture on a Dowex 1-X8 (OH^-) ion exchange column failed since the compounds were lost on the column.

Electrophoresis was performed on a Savant flatplate apparatus using Whatman No. 1 paper and 0.1 mol sodium borate at 1.5 kv, 30-35 mA for 90 min. The movement of the spot was around 17 cm toward the anode. However, separation was not accomplished.

Finally, separation was attempted using a Beckman Model 120C amino acid analyzer. As seen from the Fig. 16, the crude product showed the presence of a substance which gave a strong ninhydrin reaction but which was not indicated by the pmr spectrum (Fig. 12A). One recrystallization effectively removed this material to leave two components which are not completely separated. It is apparent from the relative intensities of the signals that the major component (equatorial *C*-methyl group) moved down the column somewhat slower than the isomer but complete separation was not achieved. The results confirm the assumption that the product is a binary mixture as is inferred in the interpretation of the pmr spectra.

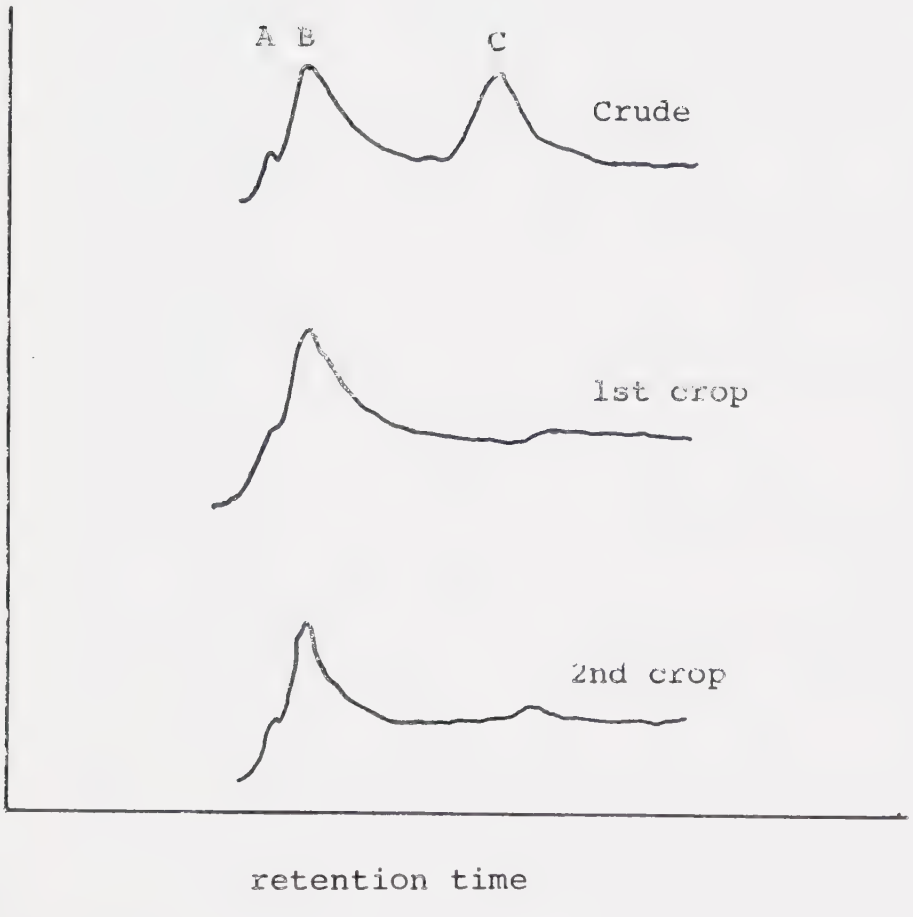

Fig. 16. Plots obtained on examining the crude product of 2,5-dideoxy-5-*C*-methylstreptamine dihydrochloride and crops from recrystallizations from ethanol-diethyl ether using an amino acid analyzer.

V. BIBLIOGRAPHY

1. H. Umezawa, M. Ueda, K. Maeda, K. Yagishita, S. Kondo, Y. Okami, R. Utahara, Y. Osato, K. Nitta, and T. Takeuchi, J. Antibiotics, Ser. A, 10, 181 (1957).
2. M. J. Cron, D. L. Evans, F. M. Palermiti, D. F. Whitehead, I. R. Hooper, P. Chu, and R. U. Lemieux, J. Am. Chem. Soc., 80, 4741 (1958).
3. K. Maeda, M. Murase, H. Mawatari, and H. Umezawa, J. Antibiotics, Ser. A, 11, 163 (1958).
4. H. Ogawa, T. Ito, S. Kondo, and S. Inoue, J. Antibiotics, Ser. A, 11, 169 (1958).
5. S. Umezawa, Y. Ito, and S. Fukatsu, J. Antibiotics, Ser. A, 11, 162 (1958).
6. (a) M. Hichens and K. L. Rinehart, Jr., J. Am. Chem. Soc., 85, 1547 (1963).
   (b) S. Tatsuoka, S. Horii, K. L. Rinehart, Jr., and T. Nakabayashi, J. Antibiotics, Ser. A, 17, 88 (1964).
7. H. Kawaguchi, Drug Action Drug Resist. Bact. 2, 45 (1975).
8. S. Umezawa, T. Tsuchiya, R. Muto, and Y. Nishimura, J. Antibiotics, 24, 274 (1971).
9. H. Umezawa, K. Iinuma, S. Kondo, M. Hamada, and K. Maeda, J. Antibiotics, 28, 340 (1975).
10. T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, Carbohyd. Res., 49, 141 (1976).

11. R. U. Lemieux and S. Koto, Tetrahedron, 30, 1933 (1974).

12. M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno, and D. Nishimura, Tetrahedron Letters, 623 (1968).

13. S. Umezawa, K. Tatsuta, and S. Koto, Bull. Chem. Soc. Japan, 42, 533 (1969).

14. S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, Bull. Chem. Soc. Japan, 42, 537 (1969).

15. S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, Bull. Chem. Soc. Japan, 42, 529 (1969).

16. T. Suami, S. Ogawa, H. Uchino, and Y. Funaki, J. Org. Chem., 40, 456 (1975).

17. T. W. Craig, G. R. Harvey, and G. A. Berchtold, J. Org. Chem., 32, 3743 (1967).

18. P. L. Barili, G. Bellucci, F. Marioni, I. Morelli, and V. Scartoni, J. Org. Chem., 37, 4353 (1972).

19. (a) A. Fürst and P. A. Plattner, Abstr. Papers Int. Congr. Pure Appl. Chem., 12th, New York, 409 (1951). (b) N. R. Williams, Advan. Carbohyd. Chem. Biochem., 25, 109 (1970).

20. D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, "Purification of Laboratory Chemicals," Pergamon Press, London, 1966.

21. L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley and Sons, Inc., New York, 1967, p. 1179.

22. L. F. Fieser and M. Fieser, *ibid*, p. 819.

23. H. Plieninger and G. Ege, Angew. Chemie, 70, 505 (1958).

24. N. A. Nelson, J. H. Fassnacht, and J. U. Piper, J. Am. Chem. Soc., 83, 206 (1961).

25. L. A. Paquette, D. E. Kuhra, J. H. Barrett, and R. J. Haluska, J. Org. Chem., 34, 2866 (1961).

26. R. L. Augustine, "Catalytic Hydrogenation," Marcel Dekker Inc., N. Y., 1965, p. 125.

27. M. J. Robins, R. Mengel, R. A. Jones, and Yves Fouron, "Nucleic Acid Related Compounds. 21. Transformation of Ribonucleoside 2',3'-*O*-Ortho Esters into Halo, Deoxy, and Epoxy Sugar Nucleosides Using Acyl Halides. Mechanism and Structure of Products," J. Am. Chem. Soc., in press.

28. A. Weissberger, "Technique of Organic Chemistry," Vol. 3, Interscience Publishers Inc., N. Y. 1956, p. 395.

29. S. Moore, D. L. Spackman and W. H. Stein, Analyt. Chem., 30, 1185 (1958).

30. R. A. Benkeser, M. L. Burrous, J. J. Hazdra, and E. M. Kaiser, J. Org. Chem., 28, 1094 (1963).

31. W. D. Kumler, P. Boikess, P. Bruck, and S. Winstein, J. Am. Chem. Soc., 86, 3126 (1964).

32. R. C. Fahey in "Topics in Stereochemistry," Vol. 3, E. L. Eliel and N. L. Allinger, Ed., Interscience, New York, 1968, p. 287.

33. J. Valls and E. Toromanoff, Bull. Soc. Chim. Fr., 758 (1961).

34. F. A. Bovey, "NMR Data Tables for Organic Compounds," Vol. 1, Interscience Publishers, New York, 1967, p. 190.

35. J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, J. Am. Chem. Soc., 92, 1338 (1970).

36. H. B. Henbest and R. A. L. Wilson, J. Chem. Soc., 1958 (1957).